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(57) Abstract

Novel multibinding compounds are disclosed. The compounds of the invention comprise 2–10 polyene macrolide antibiotic ligands covalently connected, each of said ligands being capable of binding to a ligand binding site in a cellular or viral membrane, thereby modulating the biological processes/functions thereof.

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NOVEL POLYENE MACROLIDE COMPOUNDS AND USES

BACKGROUND OF THE INVENTION

Reference to Related Application

This application claims priority to U.S. Provisional Application Serial Nos. 60/097,698 filed on August 25, 1998, 60/092,941 filed July 15, 1998 and 60/088,464 filed June 8, 1998.

Field of the Invention

This invention relates to novel therapeutic compounds that bind to sterol-containing membranes of yeast, fungi and protozoan cells and produce changes in membrane permeability and function. In particular, this invention relates to multibinding compounds comprised of 2-10 ligands covalently attached to a linker or linkers, wherein the ligands are selected from polyene macrolide antibiotics, which in their monovalent (i.e., unlinked) state are capable of insertion into biological membranes, therein forming aggregates and sterol-antibiotic complexes. The cytostatic and cytotoxic effects of these ligands are generally attributed to changes in membrane structure and integrity produced through ligand-membrane binding interactions. The manner of linking the ligands together is such that the multibinding compounds thus formed demonstrate an increased biologic and/or therapeutic effect as compared to the same number of unlinked ligands made available for membrane binding.

The compounds of this invention are particularly useful for the treatment of infectious diseases in mammals caused by pathogenic agents having binding sites

that are capable of interacting with polyene macrolide antibiotics (e.g., yeasts, fungi, protozoan parasites, viruses and bacteria). Accordingly, the invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of one or more compounds of this invention.

This invention further relates to methods of treating diseases of plants and animals mediated by fungi, parasites and other pathogenic agents comprised of macromolecular structures that are targets of polyene macrolide multibinding compounds.

Still further, this invention is directed to methods of preparing such multibinding compounds.

State of the Art

Cellular membranes comprise a selective permeability barrier to the movement of ions, small molecules and metabolites. The integrity of this barrier is essential for maintaining membrane potential, intracellular pH and concentrations of intracellular metabolites compatible with physiological processes.

Various classes of antibiotics are known that interfere with cell viability by impairing the membrane permeability barrier. Among these are the polyene macrolide antibiotics, which have potent antifungal and antiprotozoal activities. Their toxicity towards these pathogens is related to their ability to form complexes with ergosterol and structurally related sterols within the lipid bilayer, thereby altering membrane structure and promoting the leakage of cellular components. Certain polyene macrolide antibiotics are active against bacteria, viruses and tumor cells which lack ergosterol (see, e.g., Bolard, *Biochim. Biophys. Acta 864:*

257 (1986); Hartsel and Bolard,Trends in Pharmacol. Sci. 17: 445 (1996); Schaffner et al, Biochem. Pharmacol. 35:L 4110 (1986)).

The polyene macrolide antibiotics possess large (20-44-membered) lactone rings containing three to eight conjugated carbon-carbon double bonds and may also contain a sugar moiety. They are classified primarily by the number of conjugated double bonds contained in the macrolide ring moiety (see Appendix : Table 1 and Figure 1). The largest group consists of heptaenes, which can be divided into nonaromatic and aromatic groups. The aromatic heptaenes contain a side chain with an aromatic moiety, and are exemplified by candicidin (Figure 1 **(18)**). Neither the aromatic moiety nor the sugar moiety are thought to be required for antifungal activity.

Amphotericin B (Figure 1 **(2)**) is an example of a nonaromatic heptaene that is widely used clinically as an antifungal antibiotic (*Omura and Tanaka, Chapter 9, In: Macrolide Antibiotics (1984) Academic Press*).

The interaction of amphotericin with sterol-containing cell membranes has been studied extensively. These studies indicate that amphotericin increases permeability of ergosterol-containing membranes in a concentration-dependent manner. At low concentrations of amphotericin, loss of cell contents is restricted to cations and protons; however, higher concentrations or prolonged exposure results in loss of other cellular components, metabolic disruption, and cell death (*Warnock, J. Antimicrob. Chemother. 28: 27 (1991)*)

In a widely accepted model, 8-10 molecules of amphotericin assemble into a cyclic array with intercalated ergosterol molecules within a single leaflet of the

fungal cell membrane (Figure 2) (*Medoff and Kobayashi, pp. 3-33 In: The Polyenes. Antifungal Chemotherapy (D.C.E. Speller, Ed.) John Wiley & Sons (1980); Kleinberg and Finkelstein, J. Membrane Biol. 80: 257(1984)).* This amphipathic "barrel stave" superstructure encloses an aqueous pore with an estimated inner diameter of 8 Å. Alignment of two such pores, one in each of the outer and inner membrane leaflets, produces a transmembrane channel that allows for ion and small non-electrolyte passage. Alternatively, single pores may sufficiently disrupt the adjacent membrane leaflet so as to produce leakage (*Kleinberg and Finkelstein, Ibid.; Bolard, Biochim. Biophys, Acta 864: 257 (1986)*)

Amphotericin will also complex with cholesterol, the major sterol in mammalian cell membranes. However, this interaction is believed to be of lower affinity than is the interaction with ergosterol, leading to greater selectivity for fungal and protozoan cell membranes where the predominant sterol is ergosterol (*Witzke and Bittman, Biochemistry 23: 1668 (1984); Brajtburg et al, Antimicrob. Agents Chemother. 34: 183 (1990)*).

Many potent polyene macrolide antifungal agents have been isolated, but few are used clinically for the treatment of deep-seated mycotic infections because of their lack of absorption by the oral route, their insolubility in water and their parenteral toxicity. Other than amphotericin B, which has been in clinical use for more than 30 years and remains the preferred polyene macrolide antibiotic for systemic treatment of life-threatening mycotic infections, the use of other polyene macrolides (e.g., nystatin) is reserved for the topical treatment of cutaneous, oral-gastrointestinal, and vaginal yeast infections (*See, generally, Ch. 11, In: Macrolide Antibiotics, Academic Press (1984)*) .

The clinical shortcomings of amphotericin B include its consistent association with serious side effects when administered intravenously or intrathecally (i.e., nephrotoxicity, neurotoxicity). Various approaches have been used to improve the therapeutic index of amphotericin B and other polyene macrolide antibiotics. One such approach uses lipid-based polyene formulations (e.g., liposomes) to alter the tissue distribution and pharmacokinetics of the drug (*Wasan and Lopez -Berestein, Eur. J. Clin. Microbiol. Infect. Dis. 16: 81-92 (1997); Hartsel and Bolard, Trends Pharmacol. Sci. 17: 445 (1996); Rapp et al, Ann. Pharmacother. 31: 1174 (1997)*). Alternatively, various approaches have been taken to increase the water solubility of these drugs, thereby decreasing micellar formation and nonspecific membrane disruption. For example, polyene macrolide antibiotics may be complexed with cyclodextrins as described in PCT Application No. WO 89/10739, or conjugated with oligo(ethylene glycol) as described in Yamashita et al, J. Am. Chem. Soc. 117: 6249 (1995) and U.S. Patent No. 5,606,038. While these results suggest that the beneficial therapeutic effects of amphotericin B may be separated from its toxic effects, neither lipid formulation nor oligo(ethylenglycol) conjugation increases the intrinsic antifungal potency of amphotericin B. Thus there continues to exist a need for new types of drugs having improved therapeutic activities (e.g., increased potency, increased duration of action, enhanced affinity for the target binding site, increased binding affinity for ergosterol, increased binding specificity for ergosterol, decreased association with mammalian cell membranes, and increased effect against resistant organisms).

SUMMARY OF THE INVENTION

This invention is directed to novel multibinding compounds that bind to membranes of yeast, fungi, protozoa, bacteria and membrane-enveloped viruses

thereby producing membrane damage and loss of viability of these cells and viruses. The binding of these compounds to such membranes can be used to treat pathologic conditions mediated by such cells and viruses.

Accordingly, in one of its composition aspects, this invention is directed to a multibinding compound and salts thereof comprising 2 to 10 polyene macrolide antibiotic ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane.

The multibinding compounds of this invention are preferably represented by formula I:

$$(L)_p(X)_q \qquad (I)$$

wherein each L is independently selected from polyene macrolide antibiotic ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane; each X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and salts thereof. Preferably, q is $< p$.

In another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of one or more multibinding compounds or pharmaceutically acceptable salts thereof comprising 2 to 10 polyene macrolide antibiotic ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a

membrane of a cell or virus mediating a pathologic condition in a mammal or plant.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multibinding compound represented by formula I:

$$(L)_p(X)_q \qquad (I)$$

wherein each L is independently selected from polyene macrolide antibiotic ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane; each X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and salts thereof. Preferably, q is $< p$.

In one of its method aspects, this invention is directed to a method for treating a pathologic condition of a mammal or plant mediated by a cell or virus having a membrane binding site for a polyene macrolide antibiotic, which method comprises administering to said mammal or plant an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds or pharmaceutically acceptable salts thereof comprising 2 to 10 polyene macrolide antibiotic ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a membrane of said cell or virus thereby inhibiting the pathologic condition.

In another of its method aspects, this invention is directed to a method for treating a pathologic condition of a mammal or plant mediated by a cell or virus having a membrane binding site for a polyene macrolide antibiotic, which method comprises administering to said mammal or plant an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by formula I:

$$(L)_p(X)_q \qquad (I)$$

wherein each L is independently selected from polyene macrolide antibiotic ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane; each X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20 and salts thereof. Preferably, q is $< p$.

In still another method aspect, this invention is directed to methods of preparation of multibinding polyene macrolide antibiotic compounds of Formula I.

This invention is further directed to general synthetic methods for generating large libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. The diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity and polarization. The library of ligands is preferably selected to have diverse

attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

This invention is also directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a ligand or a class of ligands targeting a receptor.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

In another of its method aspects, this invention is directed to a method

for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition is preferred when a mixture of different ligands is employed to ensure heterodimeric or multimeric compounds are prepared. Concurrent addition of the ligands occurs when at least a portion of the multimer comounds prepared are homomultimeric compounds.

The assay protocols recited in (d) can be conducted on the multimeric ligand compound library produced in (c) above, or preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

In another preferred embodiment, the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heterodimeric (i.e., at least one of the ligands is different from the other ligands).

In addition to the combinatorial methods described herein, this invention provides for an interative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. Specifically, this method aspect is directed to a

method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates structures of representative polyene macrolide antibiotics. The structures are numbered to correspond to ligands of Table 1.

Figure 2 is a highly schematic drawing of an amphotericin B: ergosterol pore structure.

Figure 3 illustrates numerous reactive functional groups and the resulting bonds formed by reactions therebetween.

Figure 4 illustrates a method for optimizing the linker geometry for presentation of ligands (filled circles) in bivalent compounds:

A. phenyldiacetylene core structure

B. cyclohexane dicarboxylic acid core structure

Figure 5 shows exemplary linker "core" structures.

Figure 6 illustrates examples of multi-binding compounds comprising (A) 2 ligands, (B) 3 ligands, (C) 4 ligands, and (D) >4 ligands attached in different formatso a linker.

Figures 7 - 17 illustrate convenient methods for preparing the multibinding compounds of this invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein:

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, secondary butyl, tert-butyl, n-hexyl, n-octyl, n-decyl, n-dodecyl, 2-ethyldodecyl, tetradecyl, and the like, unless otherwise indicated.

The term "substituted alkyl" refers to an alkyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, and $-NR^aR^b$, wherein R^a and R^b may be the same or different and and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 carbon atoms. This term is exemplified by groups such as methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), the propylene isomers (e.g., $-CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2-$) and the like.

The term "substituted alkylene" refers to: (1) An alkylene group as defined above having from 1 to 5 substituents selected from the group consisting

of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocyclooxy, nitro, and $-NR_aR_b$, wherein R_a and R_b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group; (2) An alkylene group as defined above that is interrupted by 1-20 atoms independently chosen from oxygen, sulfur and NR_a-, where R_a is chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic, or groups selected from carbonyl, carboxyester, carboxyamide and sulfonyl; and (3) An alkylene group as defined above that has both from 1 to 5 substituents as defined above and is also interrupted by 1-20 atoms as defined above. Examples of substituted alkylenes are chloromethylene (-CH(Cl)-), aminoethylene ($-CH(NH_2)CH_2-$), 2-carboxypropylene isomers ($-CH_2CH(CO_2H)CH_2-$), ethoxyethyl ($-CH_2CH_2O-CH_2CH_2-$), ethylmethylaminoethyl ($-CH_2CH_2N(CH_3)CH_2CH_2-$), 1-ethoxy-2-(2-ethoxy-ethoxy)ethane ($-CH_2CH_2O-CH_2CH_2-OCH_2CH_2-OCH_2CH_2-$), and the like.

The term "alkaryl" or "aralkyl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl in which alkylene and aryl are as defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Examples of such groups are methylenemethoxy ($-CH_2OCH_3$), ethylenemethoxy ($-CH_2CH_2OCH_3$), n-propylene-iso-propoxy ($-CH_2CH_2CH_2OCH(CH_3)_2$), methylene-t-butoxy ($-CH_2-O-C(CH_3)_3$) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and

include, by way of example, methylenethiomethoxy ($-CH_2SCH_3$), ethylenethiomethoxy ($-CH_2CH_2SCH_3$), n-propylene-iso-thiopropoxy ($-CH_2CH_2CH_2SCH(CH_3)_2$), methylene-t-thiobutoxy ($-CH_2SC(CH_3)_3$) and the like.

"Alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as vinyl, prop-2-enyl, pent-3-enyl, hex-5-enyl, 5-ethyldodec-3,6-dienyl, and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, aryloxy, thioaryloxy, heteroaryloxy, thioheteroaryloxy, heterocyclooxy, thioheterocyclooxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, and, $-NR^aR^b$, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkenylene" refers to a diradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term

is further exemplified by such radicals as 1,2-ethenyl, 1,3-prop-2-enyl, 1,5-pent-3-enyl, 1,4-hex-5-enyl, 5-ethyl-1,12-dodec-3,6-dienyl, and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocyclooxy, nitro, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

"Alkynyl" refers to a monoradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as acetylenyl, prop-2-ynyl, pent-3-ynyl, hex-5-ynyl, 5-ethyldodec-3,6-diynyl, and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl,

carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocycloxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, SO_2-heterocyclic, NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkynylene" refers to a diradical of an unsaturated hydrocarbon radical, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as 1,3-prop-2-ynyl, 1,5-pent-3-ynyl, 1,4-hex-5-ynyl, 5-ethyl-1,12-dodec-3,6-diynyl, and the like.

The term "acyl" refers to the groups -CHO, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acylamino" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholine) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl).

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy,

thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, trihalomethyl, NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to a diradical derived from aryl or substituted aryl as defined above, and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group $-NH_2$

The term "substituted amino" refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" refers to the group "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted

cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl where alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring or fused rings and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

"Haloalkyl" refers to alkyl as defined above substituted by 1-4 halo groups as defined above, which may be the same or different, such as 3-fluorododecyl, 12,12,12-trifluorododecyl, 2-bromooctyl, -3-bromo-6-chloroheptyl, and the like.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl,

substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, trihalomethyl, mono-and di-alkylamino, mono- and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

The term "heteroaryloxy" refers to the group heteroaryl-O-.

The term "heteroarylene" refers to the diradical group derived from heteroaryl or substituted heteroaryl as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylene, 1,8-quinolinylene, 1,4-benzofuranylene, 2,5-pyridinylene, 1,3-morpholinylene, 2,5-indolenyl, and the like.

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and

preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, and NR^aR^b, wherein R^a and R^b may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Such heterocyclic groups can have a single ring or multiple condensed rings.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

A preferred class of heterocyclics include "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula $[-(CH_2-)_mY-]$ where m is equal to or greater than 2, and Y at each separate occurrence can be O, N, S or P. Examples of crown compounds include,

by way of example only, $[-(CH_2)_3-NH-]_3$, $[-((CH_2)_2-O)_4-((CH_2)_2-NH)_2]$ and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group derived from a heterocycle as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" refers to the group -°C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

"Alkyl optionally interrupted by 1-5 atoms chosen from O, S, or N" refers to alkyl as defined above in which the carbon chain is interrupted by O, S, or N. Within the scope are ethers, sulfides, and amines, for example 1-methoxydecyl, 1-pentyloxynonane, 1-(2-isopropoxyethoxy)-4-methylnonane, 1-(2-ethoxyethoxy)dodecyl, 2-(t-butoxy)heptyl, 1-pentylsulfanylnonane, nonylpentylamine, and the like.

"Heteroarylalkyl" refers to heteroaryl as defined above linked to alkyl as defined above, for example pyrid-2-ylmethyl, 8-quinolinylpropyl, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, optionally substituted alkyl means that alkyl may or may not be substituted by those groups enumerated in the definition of substituted alkyl.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or

three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. *See, generally,* T.W.

Greene & P.G.M. Wuts, *Protective Groups in Organic Synthesis,* 2nd Ed., 1991, John Wiley and Sons, N.Y.

The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable amino blocking groups include conventional substituents such as t-butyoxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (FMOC), allyloxycarbonyl (ALOC) and the like, which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, *t*-butyl etc. which can be removed by mild hydrolysis conditions compatible with the nature of the product.

As used herein, the terms "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith including, for example, benzene, toluene, acetonitrile, tetrahydrofuran, dimethylformamide, chloroform, methylene chloride (or dichloromethane), diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, isopropanol, tert-butanol, dioxane, pyridine, and the like. Unless specified to the

contrary, the solvents used in the reactions of the present invention are inert solvents.

"Ligand" as used herein denotes a compound that is a binding partner for a receptor and is bound thereto, for example, by complementarity. The receptor recognizes a specific region or regions of the ligand molecule designated herein as the "ligand domain". A ligand may be either capable of binding to a receptor by itself, or may require the presence of one or more non-ligand components for binding (e.g. Ca^{2+}, Mg^{2+}, a lipid molecule, a solvent molecule, and the like).

Ligands useful in this invention comprise polyene macrolide antibiotics, examples of which are given below, in Table 2 of the Appendix (including the references listed in Table 2) and in texts such as, for example, "*Macrolide Antibiotics. Chemistry, Biology and Practice*" (1984), (Omura, S. ed.), Academic Press, N.Y.

Those skilled in the art will understand that portions of the ligand structure that are not essential for molecular recognition and binding activity (i.e. that are not part of the ligand domain) may be varied substantially, replaced with unrelated structures and, in some cases, omitted entirely without affecting the binding interaction. Accordingly, it should be understood that the term ligand is not intended to be limited to polyene macrolides known to be useful as antibiotics (for example, known drugs), in that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multibinding compounds, because of the biological benefit conferred by multivalency (see below). The primary requirement for a ligand as defined herein is that it has a ligand domain, as defined above, which is available for binding to a recognition site on a cell membrane.

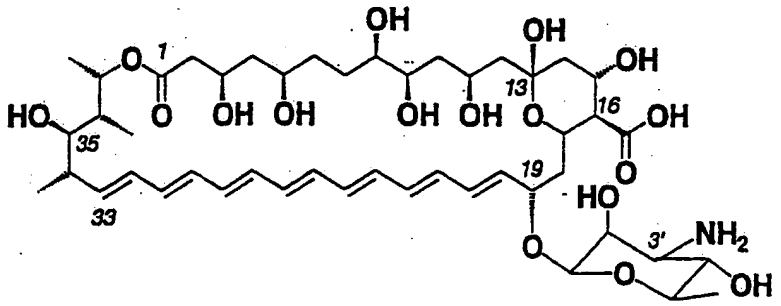
It should be understood that the term "ligand" or "ligands" is intended to include the racemic ligands as well as the individual stereoisomers of the ligands, including pure enantiomers and non-racemic mixtures thereof. The scope of the invention as described and claimed encompasses the racemic forms of the ligands as well as the individual enantiomers and non-racemic mixtures thereof.

The ligands and linkers which comprise the multibinding agents of the invention and the multibinding compounds themselves may have various stereoisomeric forms, including enantiomers and diastereomers. It is to be understood that the invention contemplates all possible sterioisomeric forms of multibinding compounds, and mixtures thereof.

The term "polyene macrolide antibiotic" refers to the chemical class of antibiotics characterized by a large (20-44 atoms) lactone ring, which contain three to eight conjugated carbon-carbon double bonds and usually a sugar moiety. These polyenes are further characterized by being amphipathic molecules having a hydrophilic surface and a hydrophobic surface. As well, most of the compounds are amphoteric, having both an ionizable carboxyl group and a basic group derived from an aromatic amine and/or an amino sugar, most commonly mycosamine. Most of these compounds are produced by soil actinomycetes, mainly of the genus *Streptomyces*. Some of the antibiotics included in this class are termed "aromatic polyenes" by virtue of their having an aliphatic side chain with an additional aromatic group attached thereto (e.g., candidicine, hamycin, auefungin, ascosin, ayfattin, azacolutin, DJ400-B, trichomycin A, levorin, heptamycin, candimycin, perimycin, vacidin A). The term "polyene macrolide antibiotic" is intended to include polyene macrolide antibiotic derivatives (e.g., semisynthetic derivatives having modifications of carboxyl, amino, hemiketal and other functional groups),

polyene macrolides lacking sugar moieties (e.g., faeriefungin, roflamycoin, roxaticin, rapamycin, dermostatin and the like) and aglycone forms of polyene macrolide antibiotics that normally contain sugars. This class of compounds exhibits diverse activities in biologic systems (e.g., fungicidal, bactericidal, herbicidal, antiviral (direct and indirect), antinematode, antiprotozoan, antitumor, antiprion, and immunomodulatory activities). Many of these activities are thought to result from the interactions of polyene macrolide antibiotics with biological membranes.

"Amphotericin B" refers to the amphipathic polyene macrolide shown below.



The term "ligand binding site'" as used herein denotes a site on a biological membrane receptor that recognizes a ligand domain of a polyene macrolide antibiotic and provides a binding partner for the ligand. The ligand binding sites of polyene macrolide antibiotics include, for example, ergosterol (and structurally related sterols) in cell membranes of fungi, *Leishmania* and *Trypanosoma,* and cholesterol (and structurally related sterols) in lipid-enveloped animal viruses and mammalian cell membranes. Some polyene macrolide antibiotics (e.g., faeriefungin) have ligand binding sites in bacterial cell membranes, which lack sterols (*Mulks, MH et al, Antimicrob. Agents Chemother. 34: 1762 (1990)).* The

ligand binding sites may be defined by monomeric or multimeric structures and may be located in one or more receptors.

Those skilled in the art will recognize that the ligand binding sites are constrained to varying degrees by their intra- and intermolecular associations. For example, cell membrane-localized binding sites may be embedded within a lipid bilayer, or associated with cytoplasmic or extracellular membrane surfaces such that their translational and rotational freedom is less than if the same sites were freely diffusable monomers in liquid solution. As well, the binding sites may be motionally restricted by the fluidity or crystallinity of the lipid domains surrounding them.

The interaction of a ligand with a ligand binding site may be capable of producing a unique biological effect, for example, agonism, antagonism, modulatory effect, and the like, or may modulate an ongoing biological effect. For example, the interaction of a polyene macrolide ligand with a ligand binding site in a biological membrane may cause the *de novo* formation of an ion channel and/or modulate the activity of a pre-existing channel (e.g., a K^+ channel). The interaction may potentiate the antifungal activity of a different class of drug, enhance clearance of a fungal organism by stimulation of the host immune system and/or directly kill the fungus.

"Multibinding agent" or "multibinding compound" refers herein to a compound that has from 2 -10 polyene macrolide ligands as defined herein (which may be the same or different) covalently bound to one or more linkers (which may be the same or different), and is capable of multivalency, as defined below.

A multibinding compound provides an improved biologic and/or therapeutic effect compared to that of the same number of unlinked ligands available for binding to ligand binding sites on the cell membrane. Without wishing to be bound by theory, the enhanced activity of the multibinding compounds of this invention is believed to arise at least in part from their ability to bind in a multivalent manner with multiple ligand binding sites in a biological membrane, which gives rise to a more favorable net free energy of binding. Multivalent binding interactions differ from collections of individual monovalent (univalent) interactions by being capable of providing enhanced biologic and/or therapeutic effect. Monovalent and multivalent binding is schematically illustrated below. Multivalent binding can amplify binding affinities and differences in binding affinities, resulting in enhanced binding specificity as well as affinity.

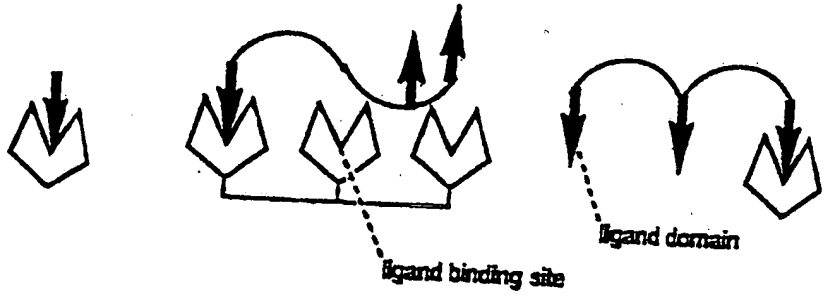
Examples of improved biologic and/or therapeutic effects include increased ligand-receptor binding interactions (e.g., increased affinity, increased ability to elicit a functional change in the target, improved kinetics), increased selectivity for the target, increased potency, increased efficacy, decreased toxicity, increased therapeutic index, improved duration of action, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multibinding compounds of the invention exhibit one or more of the foregoing effects.

More specifically, the multibinding compounds of this invention that comprise pore-forming antifungal ligands, such as amphotericin B and its congeners, will promote the efficient formation of pores in ergosterol-containing membranes, and thereby increase the leakage of fungal cell components and potencies and rates of killing of fungal organisms when compared with the same

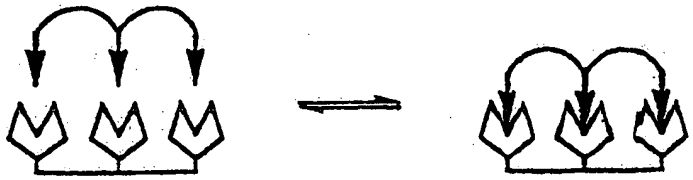
number of unlinked ligands made available for univalent binding. The biologic and/or therapeutic advantages of such compounds may include increased affinity for ergosterol, increased specificity for ergosterol-containing membranes over cholesterol-containing membranes, increased antifungal potency, increased duration of action and postantibiotic effect, increased therapeutic index, and/or increased activity against amphotericin-resistant organisms. It should be understood however, that the scope of this invention is not limited to polyene macrolide antibiotics whose mechanism of action involves pore formation. The biologic and/or therapeutic advantages of multibinding polyene macrolide compounds can be significant where the desired effect is not known to involve pore formation (e.g., bactericidal activity, antifungal activity of polyene macrolides smaller than heptaenes) or sterol complexation (e.g., enhancement of host resistance to infectious agents, potentiation of antiviral compounds, antiprion activity, and the like). *See, generally, reviews by Bolard, Biochim.Biophys.Acta 864: 257-304 (1986) Hartsel & Bolard, TIPS 17: 445-449 (1996).*

"Multimeric compounds" refer to multimers that are connected through at least one linker which may or may not possess multibonding properties.

"Univalency" or "monovalency" as used herein refers to a single binding interaction between one ligand with one ligand binding site as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibits univalency when only one ligand of that compound interacts with a ligand binding site. Examples of univalent interactions are depicted below.



"Multivalency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands, which may be the same or different, and two or more corresponding ligand binding sites, which may be the same or different. An example of trivalent binding is depicted below for illustrative purposes.



It should be understood that not all compounds that contain multiple copies of a ligand attached to a linker or linkers necessarily exhibit the phenomena of multivalency, i.e., that the biologic and/or therapeutic effect of the multi-binding compound is greater than the biologic and/or therapeutic effect of the same number of unlinked ligands available for binding to the ligand binding sites. For multivalency to occur, the ligand domains of the ligands that are linked together must be presented to their cognate ligand binding sites by the linker in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multibinding interaction.

The term "linker" or "linkers", identified where appropriate by the symbol X, refers to a group or groups that covalently link(s) from 2 to 10 ligands (as defined above) in a manner that provides a compound capable of multivalency. The linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. The term "linker" includes

everything that is not considered to be part of the ligand, e.g., ancillary groups such as solubilizing groups, lipophilic groups, groups that alter pharmacodynamics or pharmacokinetics, groups that modify the diffusability of the multi-binding compound, groups that attach the ligand to the linker, groups that aid the ligand-orienting function of the linker, for example, by imparting flexibility or rigidity to the linker as a whole, or to a portion thereof, and so on.

The extent to which the previously discussed enhanced multivalent binding is realized in this invention depends upon the efficiency with which the linker or linkers that joins the polyene macrolide ligands presents them to their receptors in the target membranes. Beyond presenting ligands for multivalent interactions with receptors, the linker spatially constrains these interactions to occur within dimensions defined by the linker. The preferred linker length will vary depending upon the distance between adjacent ligand binding sites, and the geometry, flexibility and composition of the linker. The length of the linker will preferably be in the range of about 2Å to about 100Å, more preferably from about 2Å to about 50Å, and even more preferably from about 4Å to about 20Å. The term "linker" does not however cover solid inert supports such as beads, glass particles, rods, and the like.

The biological activity of the multibinding polyene macrolide antibiotic compound is highly sensitive to the geometry, composition, size, flexibility or rigidity, the presence or absence of anionic or cationic charge, the relative hydrophobicity/ hydrophilicity, and similar properties of the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the compound. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands for binding to the receptors to permit

multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multibinding compound.

For example, polyene macrolide antibiotics may be linked together so as to increase the efficiency of transmembrane pore formation in fungal membranes. According to the well-known "barrel stave model" (see Figure 2 of the Appendix), the amphotericin B-associated membrane pore structures in each leaflet of the membrane bilayer comprise 8-10 subunits of amphotericin B oriented with their long axes perpendicular to the membrane surface and interleaved in a head-to-tail fashion around the perimeter of the pores with sterols intercalated between the subunits (not shown). The maximum distance between subunits across the pores is estimated to be about 20 Å. (*Medoff and Kobayashi, pp. 3-33 In: The Polyenes. Antifungal Chemotherapy (D.C.E. Speller, Ed.) John Wiley & Sons (1980); Kleinberg and Finkelstein, J. Membrane Biol. 80: 257(1984.* As is discussed below, the structural features of the linker (e.g., length, geometry, valency, size, flexibility, chemical composition and orienting capabilities) are varied to prepare multibinding compounds with the desired biological activities.

The linker may be biologically "neutral," i.e., not itself contribute any additional biological activity to the multibinding compound, or it may be chosen to further enhance the biological activity of the compound. For example, linkers (frameworks) that increase the solubility and critical micelle concentrations of polyene macrolide multibinding compounds relative to the unlinked ligands will decrease non-specific membrane-disruption and increase the therapeutic index of these compounds as antifungal agents.

The linker is attached to the ligand at a position that retains ligand domain-receptor binding and orients the ligand domain of the ligand for binding to the ligand binding site.

The relative orientation in which the ligand domains are displayed depends both on the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable substitutions can be made on a ligand is typically based on prior knowledge of structure-activity relationships of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., NMR and CD are often used to investigate the interactions of polyene macrolide antibiotics with sterols and lipids). Such positions and synthetic protocols for linkage are well known in the art and can be determined by those with ordinary skill in the art.

The optimal geometry and composition of linker frameworks are based upon the properties of their intended receptors. Different linker frameworks can be designed to provide preferred orientations of the ligands in the multibinding compounds of this invention, using systematic iterative spatial searching strategies known to those skilled in the art of molecular design.

Following attachment of a ligand to the linker or a significant portion thereof (e.g., 2-10 atoms of linker), the linker-ligand conjugate may be tested for retention of activity in a relevant assay system (see Testing and Examples below for representative assays).

Properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multi-

binding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention.

As previously noted, in a preferred embodiment of this invention, linkers with overall hydrophilic properties are used to enhance the water solubility of the multibinding compounds by comparison with the monovalent ligand. Thus the inclusion of ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols, (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligosaccharides, etc.) carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like will be useful. In particularly preferred embodiments, the ancillary group will contain a small number of repeating ethylene oxide ($-CH_2CH_2O-$) units.

The hydrophobic/hydrophilic characteristics of the linker can readily be controlled by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ($H_2N(CH_2)_6NH_2$) or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines" (class of surfactants).

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the compounds of Formula I is also within the scope of this invention. For example, lipophilic

groups may be added to modulate interactions between the multi-binding compound and biological membranes where this is desired. Lipophilic groups preferred for use in the linkers of this invention include, but are not limited to, alkyl, aryl, heteroaryl and substituted aralkyl groups containing from 6-18 carbons. Other preferred lipophilic groups include fatty acid derivatives. In a preferred embodiment of this invention, a relatively hydrophobic linker is used to mask or otherwise alter functional groups that contribute to the insolubility of the monovalent ligand, thus increasing the total solubility of the multibinding compound.

Also within the scope of this invention is the use of ancillary groups that result in the multibinding polyene macrolide compounds being incorporated into a liposome or lipid complex (for examples of useful lipid-drug delivery systems for amphotericin, see *Rapp et al, The Annals of Pharmacotherapy 31: 1174-1186 (1997); Wasan & Lopez-Berestein, Eur. J. Clin. Microbiol. Infect. Dis. 16: 81-92 (1997)*. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine,

lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine and dilinoleoylphosphatidylcholine. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families, are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker, or bonds between the linker and the ancillary group(s), or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational freedom is restrained by the presence of rings and/or π-bonds, for example, aryl, heteroaryl and heterocyclic groups. Other groups that can impart rigidity include polypeptide groups such as oligo- or polyproline chains. In preferred embodiments, rigidity (entropic control) is imparted by the presence of alicyclic (e.g., cycloalkyl), aromatic and heterocyclic groups. In other preferred embodiments, this comprises one or more six-membered rings.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the linker into a configuration affording the maximum distance between each of the like charges. The addition of ancillary groups which are charged, or alternatively, protected groups that bear a latent charge which is unmasked, following addition to the linker, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon ð-bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

The use of rigid frameworks with appropriate distance and geometric constraints is envisioned for constructing multibinding compounds that serve to nucleate multiple pores in close proximity for the purpose of enhancing leakage of fungal cell contents and killing activity. Two distinct types of adjacencies are possible, "side-by-side" in the same leaflet of the cell membrane, or "bottom-to-bottom" spanning both membrane leaflets.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art.

The linker or linkers, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multi-binding compound. At present, it is preferred that the multibinding compound is a bivalent compound in which two ligands are covalently linked.

The ligands are covalently attached to the linker or linkers using conventional chemical techniques. The reaction chemistries resulting in such linkage are well known in the art and involve the use of reactive functional groups

present on the linker and ligand. Preferably, the reactive functional groups on the linker are selected relative to the functional groups available on the ligand for coupling, or which can be introduced onto the ligand for this purpose. Again, such reactive functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker. Figure 3 illustrates numerous reactive functional groups and the resulting bonds formed by reaction therebetween. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March, *Advanced Organic Chemistry*, 4th Ed., (Wiley-Interscience, N.Y., 1992).

The linker is attached to the ligand at a position that retains ligand domain-ligand binding site interaction and specifically which permits the ligand domain of the ligand to orient itself to bind to the ligand binding site. Such positions and synthetic protocols for linkage are well known in the art. The term linker embraces everything that is not considered to be part of the ligand.

The relative orientation in which the ligand domains are displayed depends both on the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable

substitutions can be made on a ligand is typically based on prior knowledge of structure-activity relationships (SAR) of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., X-ray crystallography, NMR, and the like). Such positions and synthetic protocols for linkage are well known in the art and can be determined by those with ordinary skill in the art (*see* Methods of Preparation .) Following attachment of a ligand to the linker or linkers, or to a significant portion thereof (e.g., 2-10 atoms of linker), the linker-ligand conjugate may be tested for retention of activity in a relevant assay system (*see* Utility and Testing below for representative assays).

At present, it is preferred that the multibinding compound is a bivalent compound in which two ligands are covalently linked, or a trivalent compound, in which three ligands are covalently linked. Linker design is further discussed under Methods of Preparation.

"Potency" as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its receptor. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multi-binding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g. in an *in vitro* or *in vivo* assay, in an appropriate animal model). The finding that the multi-binding agent produces an equivalent biologic or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g. on a per weight, per mole or per ligand basis) is indicative of enhanced potency.

"Selectivity" or "specificity" is a measure of the binding preferences of a ligand for different receptors. The selectivity of a ligand with respect to its target receptor relative to another receptor is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex) or, in cases where a biological effect is observed below the K_d , the ratio of the respective EC_{50}s (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct receptors).

The term "treatment" refers to any treatment of a disease or condition in a mammal, particularly a human, and includes:

1. preventing the disease or condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the pathologic condition;

2. inhibiting the disease or condition, i.e., arresting its development;

3. relieving the disease or condition, i.e., causing regression of the disease or condition; or

4. relieving the symptoms resulting from the disease or condition without addressing the underlying disease or condition.

The phrase "disease or condition which is modulated by treatment with a multibinding compound" covers all disease states and/or conditions that are generally acknowledged in the art to be usefully treated with a polyene macrolide antibiotic ligand in general, and those disease states and/or conditions that have been found to be usefully treated by a specific multi-binding compound of our invention, i.e., the compounds of Formula I. Such disease states include, by way of example only, invasive aspergillosis, blastomycosis, coccidioidmycosis,

cryptococcosis, histoplasmosis, mucormycosis, and sporotrichosis, candidiasis, leishmaniasis, plant diseases attributable to molds (e.g., Aspergillus flavus and Fusarium spp.), microbial infections, and diseases caused by lipid-enveloped viruses. Conditions that reduce host resistance to microbial infections are also intended to be covered.

The term "effective amount" refers to that amount of multi-binding compound that is sufficient to effect treatment, as defined above, when administered to a mammal or plant crop in need of such treatment. The effective amount will vary depending upon the host and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "pharmaceutically acceptable excipient" is intended to include vehicles and carriers capable of being coadministered with a multi-binding compound to facilitate the performance of its intended function (e.g., cyclodextrins, lipid formulations, liposomes). The use of such media for pharmaceutically active substances is well known in the art. Examples of such vehicles and carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. Any other conventional carrier suitable for use with the multi-binding compounds also falls within the scope of the present invention.

METHODS OF PREPARATION

Linkers

The linker or linkers, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multibinding

compound. The biological activity of the multibinding compound is highly sensitive to the geometry, composition, size, length, flexibility or rigidity, the presence or absence of anionic or cationic charge, the relative hydrophobicity/hydrophilicity, and similar properties of the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the compound. The linker may be biologically "neutral," i.e., not itself contribute any additional biological activity to the multibinding compound, or it may be chosen to further enhance the biological activity of the compound. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands for binding to the receptors to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multibinding compound.

For example, different orientations of ligands can be achieved by varying the geometry of the framework (linker) by use of mono- or polycyclic groups, such as aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). The optimal geometry and composition of frameworks (linkers) used in the multibinding compounds of this invention are based upon the properties of their intended receptors. For example, it is preferred to use rigid cyclic groups (e.g., aryl, heteroaryl), or non-rigid cyclic groups (e.g., cycloalkyl or crown groups) to reduce conformational entropy when such may be necessary to achieve energetically coupled binding.

Different frameworks can be designed to provide preferred orientations of the ligands. The identification of an appropriate framework geometry for ligand

domain presentation is an important first step in the construction of a multi binding agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process. Figures 4A and 4B illustrate a useful strategy for determining an optimal framework display orientation for ligand domains and can be used for preparing the bivalent compounds of this invention. Various alternative strategies known to those skilled in the art of molecular design can be substituted for the one described here.

As shown in Figures 4A and 4B, the ligands (shown as filled circles) are attached to a central core structure such as phenyldiacetylene (Panel A) or cyclohexane dicarboxylic acid (Panel B). The ligands are spaced apart from the core by an attaching moiety of variable lengths *m* and *n*. If the ligand possesses multiple attachment sites (see discussion below), the orientation of the ligand on the attaching moiety may be varied as well. The positions of the display vectors around the central core structures are varied, thereby generating a collection of compounds. Assay of each of the individual compounds of a collection generated as described will lead to a subset of compounds with the desired enhanced activities (e.g., potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will suggest a framework orientation that favors the properties desired.

The process may require the use of multiple copies of the same central core structure or combinations of different types of display cores. It is to be noted that core structures other than those shown here can be used for determining the optimal framework display orientation of the ligands. The above-described

technique can be extended to trivalent compounds and compounds of higher-order valency.

A wide variety of linkers is commercially available (e.g., Chem Sources USA and Chem Sources International; the ACD electronic database; and Chemical Abstracts). Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art, and as described below. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatics, heterocyclics, ethers, lipids, cationic or anionic groups, or a combination thereof.

Examples are given below and in Figure 5, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto the linker enhances the hydrophilicity and water solubility of the multibinding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further, PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

In preferred embodiments, rigidity (entropic control) is imparted by the presence of alicyclic (e.g., cycloalkyl), aromatic and heterocyclic groups. In other preferred embodiments, this comprises one or more six-membered rings. In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl, or a macrocyclic ring such as, for example, a crown compound.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art.

Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention. In certain cases, the antigenicity of a multibinding compound may be eliminated or reduced by use of groups such as, for example, poly(ethylene glycol).

The Compounds of Formula I

As explained above, the multi-binding compounds described herein comprise 2-10 polyene macrolide antibiotic ligands attached covalently to a linker that links the ligands in a manner that allows their multivalent binding to ligand binding sites of biological membranes. The linker spatially constrains these interactions to occur within dimensions defined by the linker. This and other factors increase the biologic and/or therapeutic effect of the multi-binding compound as compared to the same number of ligands used in monobinding form.

The compounds of this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where L, X, p and q are as defined above. This is

intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is provided below.

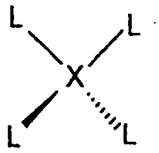
As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain and/or at any intermediate position thereof.

The simplest and most preferred multibinding compound is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different and X is the linker. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as is X. However, a trivalent compound can also comprise three ligands attached to a central core, and thus be represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group. Tetravalent compounds can be represented in a linear array:

L-X-L-X-L-X-L,

or a branched array:

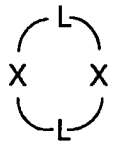
i.e., a branched construct analogous to the isomers of butane (*n*-butyl, *iso*-butyl, *sec*-butyl, and *t*- butyl) or a tetrahedral array, e.g.



where X and L are as defined herein. Alternatively, it could be represented as an alkyl, aryl or cycloalkyl derivative as described above with four (4) ligands attached to the core linker.

The same considerations apply to higher multibinding compounds of this invention containing from 5-10 ligands. However, for multibinding agents attached to a central linker such as an aryl, cycloalkyl or heterocyclyl group, or a crown compound, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

The formula $(L)_p(X)_q$ is also intended to represent a cyclic compound of formula $(\text{-L- X-})_n$,where n is 2-10. Certain of the above described compounds may alternatively be represented as cyclic chains of the form:



and variants thereof.

All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$. Examples of bivalent and higher-order valency compounds of this invention are provided in Figures 6A to 6D.

With the foregoing in mind, a preferred linker may be represented by the following formula:

$$-X^a\text{-}Z\text{-}(Y^a\text{-}Z)_m\text{-}Y^b\text{-}Z\text{-}X^a\text{-}$$

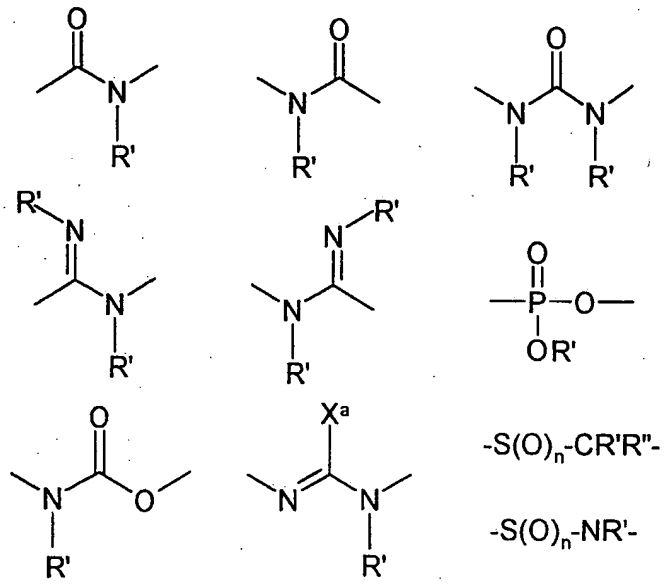
in which:

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of:



-S-S- or a covalent bond;

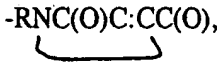
in which:

n is 0, 1 or 2; and

R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.

Other preferred linkers include N-substituted maleimide represented by the formula:



where R is as defined above,

and aminoacyl linkers of the formula:

-C(O)CR R'NR"-

where R, R' and R" are defined as above.

In one embodiment of this invention, the linker (i.e., X, X' or X") is selected from those shown in Table 3:

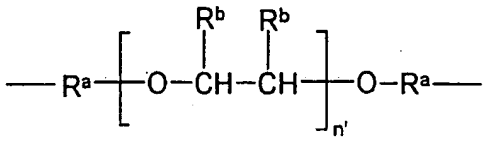
Table 3

Representative Linkers

Linker
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_3$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_4$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_5$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_6$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_7$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_8$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_9$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_{10}$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_{11}$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_{12}$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,2-phenyl

Linker
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,3-phenyl
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,4-phenyl
-HN-$(CH_2)_2$-NH-C(O)-Z-O-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,4-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-$(CH_2)_8$-CH_3)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-O-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 5-(*n*-octadecyloxy)-1,3-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-Z)-C(O)-NH-$(CH_2)_2$-NH- where Z is 4-biphenyl
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 5-(n-butyloxy)-1,3-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_8$-*trans*-(CH=CH)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-$(CH_2)_{12}$-CH_3)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-Z)-C(O)-NH-$(CH_2)_2$-NH- where Z is 4-(n-octyl)-phenyl
-HN-(CH_2)-Z-O-$(CH_2)_6$-O-Z-(CH_2)-NH- where Z is 1,4-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-NH-C(O)-$(CH_2)_3$-C(O)-NH-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-Ph)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-N+($(CH_2)_9$-CH_3)(CH_2-C(O)-NH-$(CH_2)_2$-NH_2)-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-N($(CH_2)_9$-CH_3)-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-NH-C(O)-$(CH_2)_3$-C(O)-NH-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 5-hydroxy-1,3-phenyl

In another embodiment of this invention, the linker (i.e., X, X' or X") has the formula:



wherein

each R^a is independently selected from the group consisting of a covalent bond, alkylene, substituted alkylene and arylene;

each R^b is independently selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

n' is an integer ranging from 1 to about 20.

In view of the above description of the linker, it is understood that the term "linker" when used in combination with the term "multibinding compound" includes both a covalently contiguous single linker (e.g., L-X-L) and multiple covalently non-contiguous linkers (L-X-L-X-L) within the multibinding compound.

As was previously discussed, the linker or linkers can be attached to different positions on the ligand molecule to achieve different orientations of the ligand domains and thereby facilitate multivalency. Preferred positions of attachment suggested by known SAR are illustrated in the reaction schemes described herein.

Certain polyene macrolide ligands may be chiral and exhibit stereoselectivity. The most active enantiomers are preferably used as ligands in

the multibinding compounds of this invention. The chiral resolution of enantiomers is accomplished by well known procedures that result in the formation of diastereomeric derivatives or salts, followed by conventional separation by chromatographic procedures or by fractional crystallization (*see, e.g.*, Bossert, et al., *Angew. Chem. Int. Ed.*, **20**:762-769 (1981) and U.S. Patent No. 5,571,827 and references cited therein). Enantiomers are also accessible by asymmetric synthesis. Linkers can be achiral, chiral or otherwise stereomeric.

The present invention also relates to all multibinding compounds having chiral and otherwise stereoisomeric multivalent constituents.

The linker to which the ligands or ligand precursors are attached comprises a "core" molecule having two or more functional groups with reactivity that is complementary to that of the functional groups on the ligand. Figure 5 illustrates the diversity of "cores" that are useful for varying the linker size, shape, length, orientation, rigidity, acidity/basicity, hydrophobicity/hydrophilicity, hydrogen bonding characteristics and number of ligands connected. This pictorial representation is intended only to illustrate the invention, and not to limit its scope to the structures shown. In the Figures and reaction schemes that follow, a solid circle is used to generically represent a core molecule. The solid circle is equivalent to a linker as defined above after reaction.

The same considerations apply to higher multibinding compounds of this invention containing from 5-10 ligands. However, for multibinding agents attached to a central linker such as an aryl , cycloalkyl or heterocyclyl group, or a crown compound, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for

example, a benzene ring could not directly accommodate more than 6 ligands, whereas a saturated and/or multi-ring linker (e.g.,cyclohexyl, cyclooctyl, biphenyl) could accommodate a larger number of ligands.

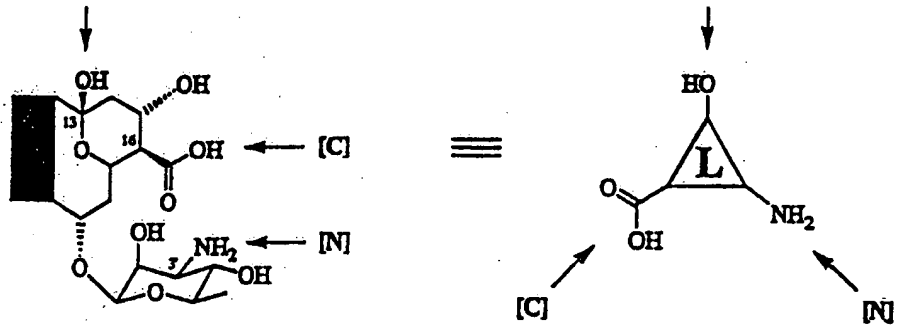
Preparation of Compounds of Formula I

The ligands are covalently attached to the linker using conventional chemical techniques. The reaction chemistries resulting in such linkage are well known in the art and involve the coupling of reactive functional groups present on the linker and ligand. Preferably, the reactive functional groups on the linker are selected relative to the functional groups available on the ligand for binding, or which can be introduced onto the ligand for binding. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as *J. March, "Advanced Organic Chemistry", 4th Edition (Wiley- Interscience, N.Y., 1992)*. Examples of the chemistry for connecting ligands by a linker are shown in Figure 3, where R_1 and R_2 represent a ligand and/or the linking group.

As indicated above, the simplest (and preferred) construct is a bivalent compound which can be represented as L-X-L, where L is a polyene macrolide antibiotic ligand that is the same or different at each occurrence, and X is the linker. Accordingly, examples of the preparation of a bivalent ligand are given below as an illustration of the manner in which multi-binding compounds of Formula I are obtained.

In the reaction schemes that follow, only the portion of the polyene macrolide ligand that is involved in the linking reaction is shown. For example, structure (1) below represents a ligand such as amphotericin B and other polyene

macrolide antibiotics with a similar substructure that includes hemiketal, carboxyl and amino groups of an amino sugar.

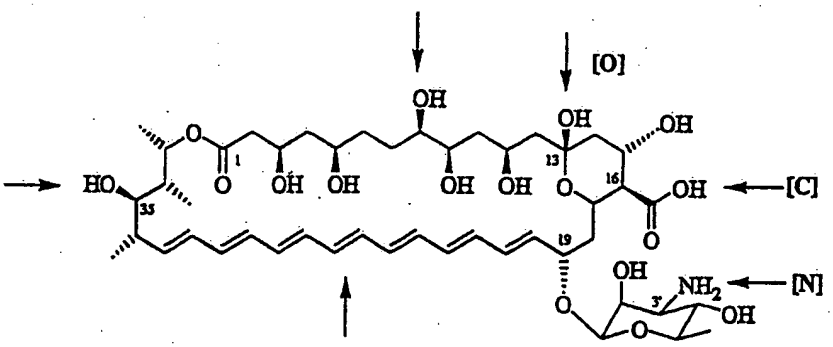


(1) (1)

The examples are intended to illustrate preferred linking strategies, and are intended to apply to any polyene macrolide antibiotic ligand that includes, or can be functionalized with groups compatible with the chosen linker.

Ligands can be linked at various positions to achieve different orientations of the ligand domains, and thereby facilitate multivalency, as was previously discussed. By way of illustration, the positions that may be used for linking amphotericin B are indicated by arrows in the Figure shown below.

Based on known Structure Activity Relationship (SAR) data, presently preferred positions for linking are the carboxy [C] group, the amine [N] group, and the hemiketal [O] group.



A particularly preferred linkage is between the amino group of a first ligand and a carboxy group of a second ligand (i.e., a "head- to -tail" linkage).

In some cases, it is preferred to link ligands directly, using the functionality already present in the monovalent ligand. In other cases, it is preferred to accomplish linking indirectly by first preparing an intermediate that is further reacted to form the multibinding agents of the invention. In some cases, it may be necessary to protect portions of the ligand that are not involved in linking reactions. Protecting groups for this purpose are well known to those skilled in the art. Various coupling reactions can be used, some of which are exemplified in the reaction schemes that follow. One skilled in the art will appreciate that synthetically equivalent coupling reactions can be substituted for those illustrated herein.

Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which possess multibinding properties.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to

toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or are elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in momomeric form.

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets.

For example, amphotericin and pneumocandin may be joined to a linker through attachment points which do not abrogate the binding affinity of the

monomeric ligands for their respective receptor sites. Both target species are present in the fungal cell plasma membrane. The amphotericin interacts with ergosterol and pneumocandin interacts with β-D glucan synthase. If the pneumocandin unit enhances the activity of the amphotericin at the ergosterol target and/or amphotericin enhances the activity of the pneumocandin at the β-D-glucan synthase, then the expected activity will be above and beyond that of the combination of the monomeric species

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically inocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and

specificities more than sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available (such as 7TM G-protein coupled receptors), one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding sites reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around

a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarizability, polaritability acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

Having chosen a set of *n* ligands (*n* being determined by the sum of the number of different attachment points for each ligand chosen) and *m* linkers by the process outlined above, a library of (*n*!)*m* candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalities on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial library is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values are determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, are also determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data are determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate

information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries are then generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is

able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

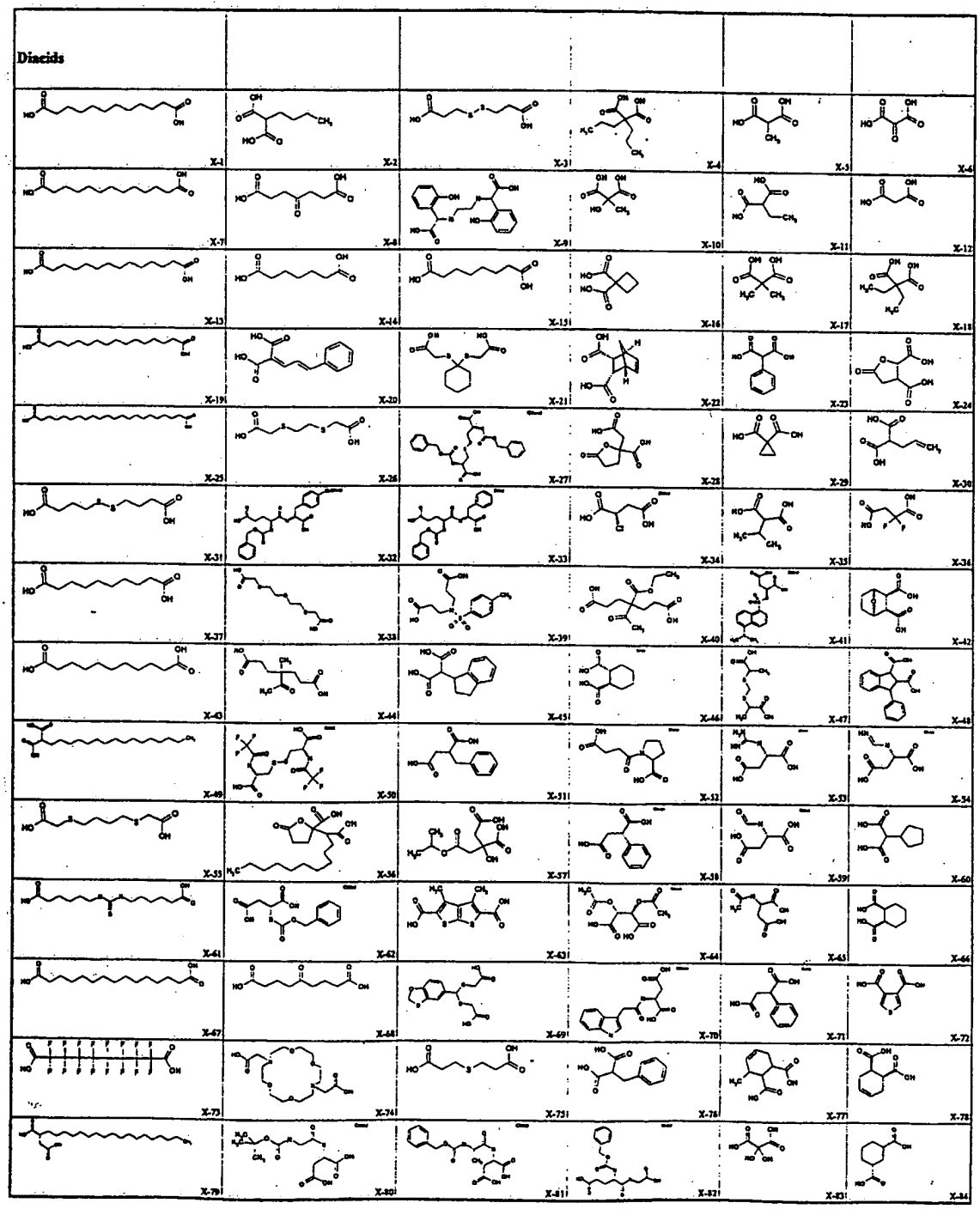
To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates,diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

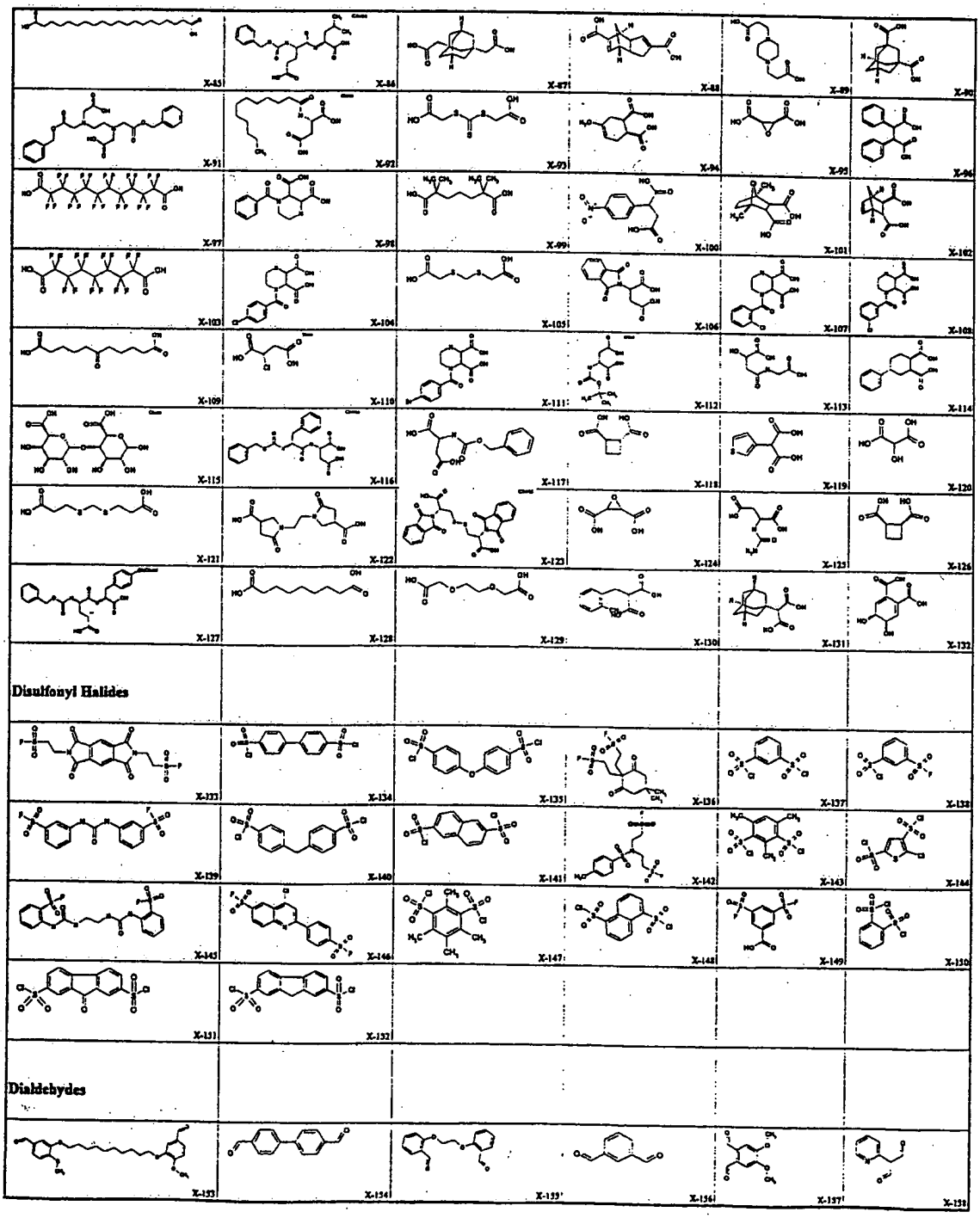
COMPLEMENTARY BINDING CHEMISTRIES

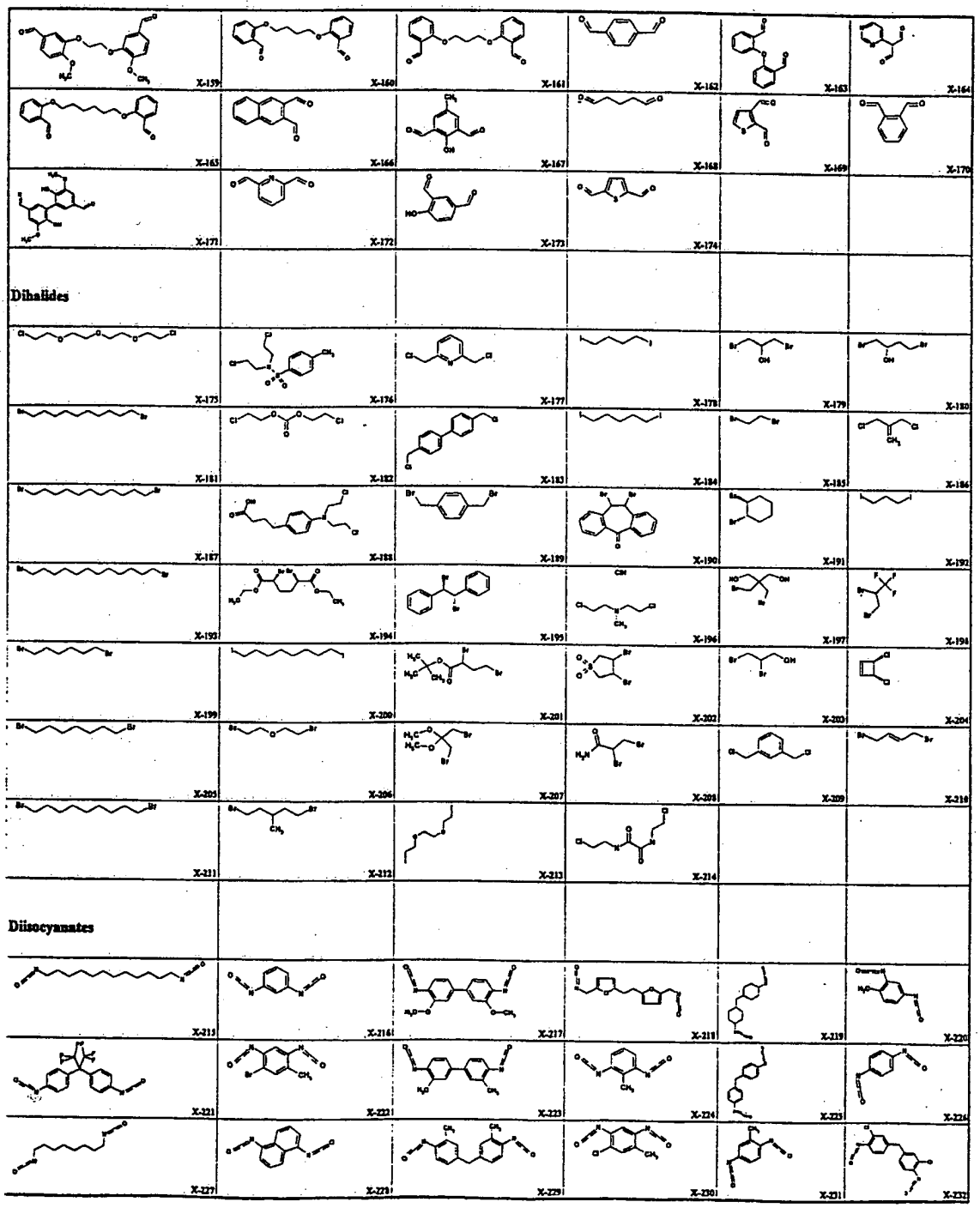
First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-aminohydroxy
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_3$	amine
ketone	amine/$NaCNBH_3$	amine
amine	isocyanate	carbamate

Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below.

Diacids




Disulfonyl Halides
Dialdehydes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X-172
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Dihalides
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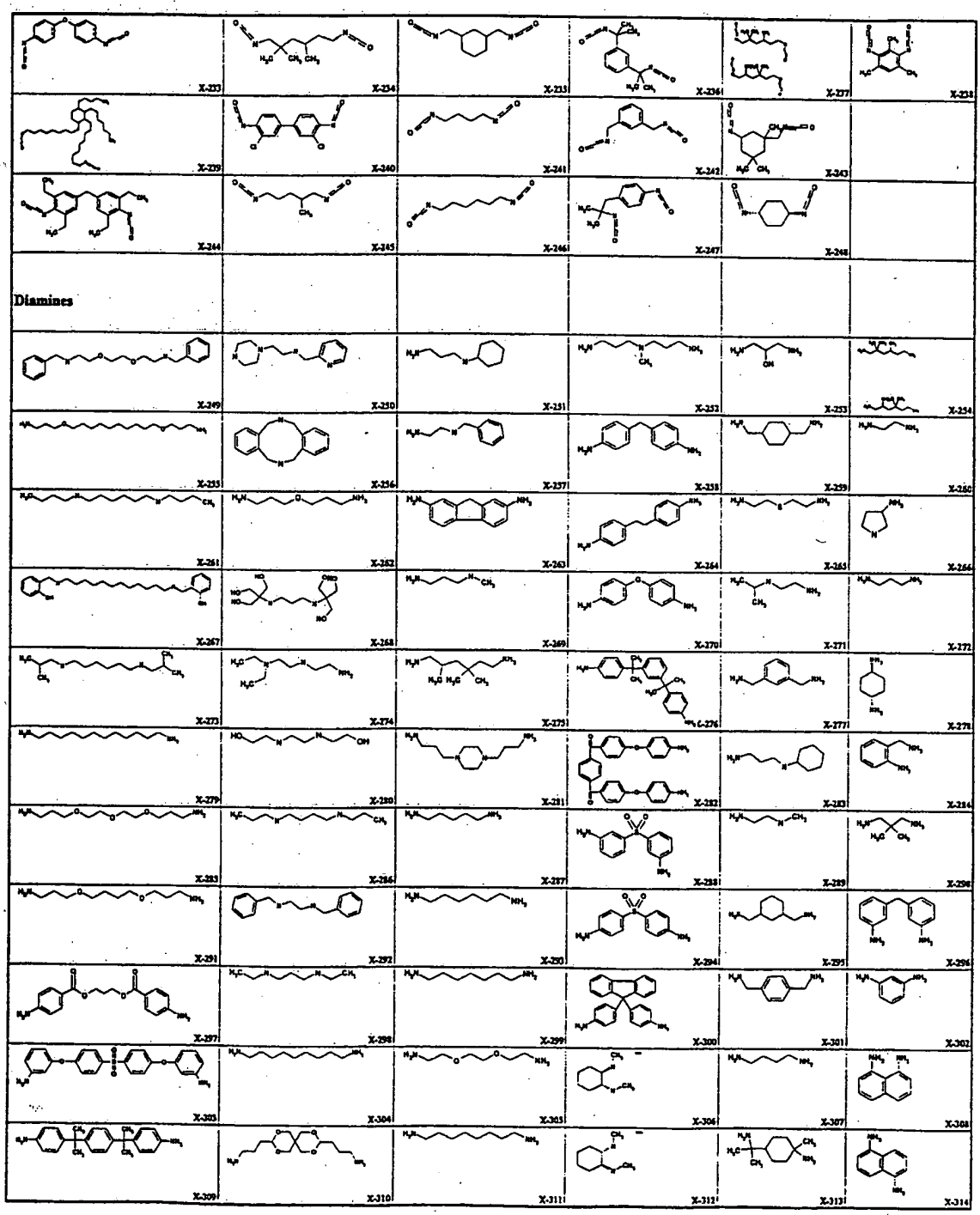
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Diamines
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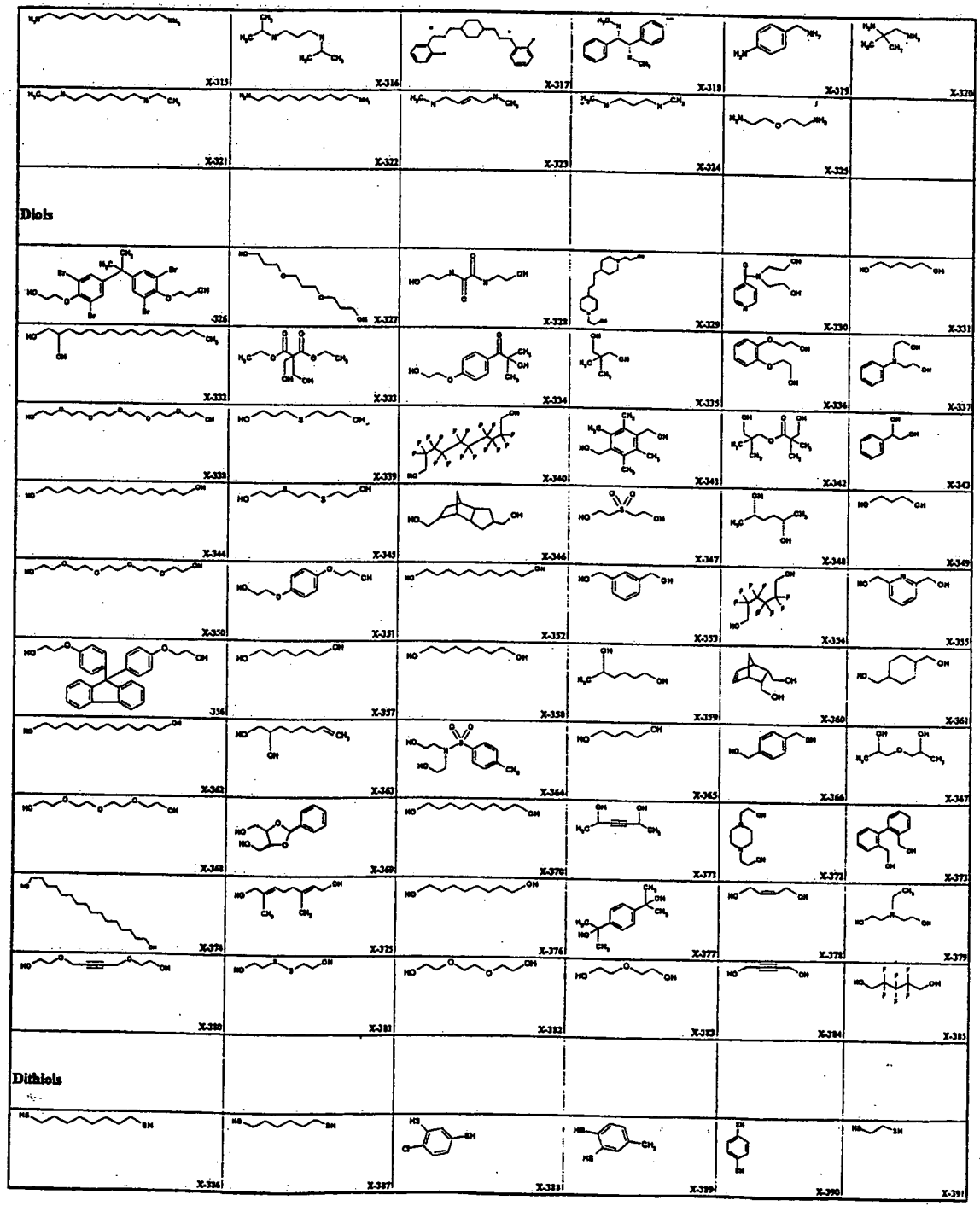
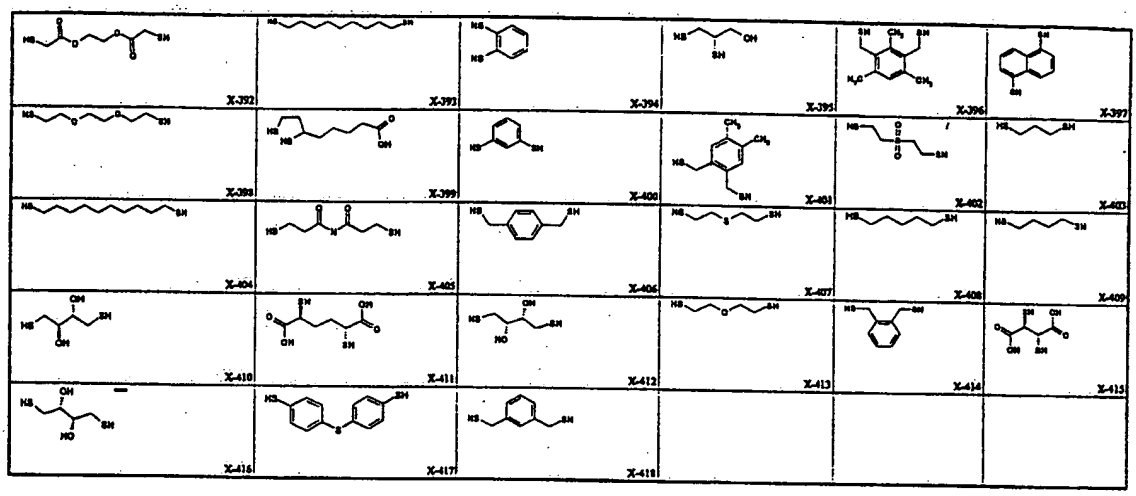


Diols

Dithiols



Representative ligands for use in this invention include, by way of example, L-1 through L-3 as identified above.

For example, L-1 can be amphotericin B;
L-2 can be nystatin A; and
L-3 can be lienomycin.

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-3 above and the second ligand and linker is selected from the following:

L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-

L-1/X-91- L-1/X-92- L-1/X-93- L-1/X-94- L-1/X-95- L-1/X-96-
L-1/X-97- L-1/X-98- L-1/X-99- L-1/X-100- L-1/X-101- L-1/X-102-
L-1/X-103- L-1/X-104- L-1/X-105- L-1/X-106- L-1/X-107- L-1/X-108-
L-1/X-109- L-1/X-110- L-1/X-111- L-1/X-112- L-1/X-113- L-1/X-114-
L-1/X-115- L-1/X-116- L-1/X-117- L-1/X-118- L-1/X-119- L-1/X-120-
L-1/X-121- L-1/X-122- L-1/X-123- L-1/X-124- L-1/X-125- L-1/X-126-
L-1/X-127- L-1/X-128- L-1/X-129- L-1/X-130- L-1/X-131- L-1/X-132-
L-1/X-133- L-1/X-134- L-1/X-135- L-1/X-136- L-1/X-137- L-1/X-138-
L-1/X-139- L-1/X-140- L-1/X-141- L-1/X-142- L-1/X-143- L-1/X-144-
L-1/X-145- L-1/X-146- L-1/X-147- L-1/X-148- L-1/X-149- L-1/X-150-
L-1/X-151- L-1/X-152- L-1/X-153- L-1/X-154- L-1/X-155- L-1/X-156-
L-1/X-157- L-1/X-158- L-1/X-159- L-1/X-160- L-1/X-161- L-1/X-162-
L-1/X-163- L-1/X-164- L-1/X-165- L-1/X-166- L-1/X-167- L-1/X-168-
L-1/X-169- L-1/X-170- L-1/X-171- L-1/X-172- L-1/X-173- L-1/X-174-
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L-1/X-187- L-1/X-188- L-1/X-189- L-1/X-190- L-1/X-191- L-1/X-192-
L-1/X-193- L-1/X-194- L-1/X-195- L-1/X-196- L-1/X-197- L-1/X-198-
L-1/X-199- L-1/X-200- L-1/X-201- L-1/X-202- L-1/X-203- L-1/X-204-
L-1/X-205- L-1/X-206- L-1/X-207- L-1/X-208- L-1/X-209- L-1/X-210-
L-1/X-211- L-1/X-212- L-1/X-213- L-1/X-214- L-1/X-215- L-1/X-216-
L-1/X-217- L-1/X-218- L-1/X-219- L-1/X-220- L-1/X-221- L-1/X-222-
L-1/X-223- L-1/X-224- L-1/X-225- L-1/X-226- L-1/X-227- L-1/X-228-
L-1/X-229- L-1/X-230- L-1/X-231- L-1/X-232- L-1/X-233- L-1/X-234-
L-1/X-235- L-1/X-236- L-1/X-237- L-1/X-238- L-1/X-239- L-1/X-240-
L-1/X-241- L-1/X-242- L-1/X-243- L-1/X-244- L-1/X-245- L-1/X-246-
L-1/X-247- L-1/X-248- L-1/X-249- L-1/X-250- L-1/X-251- L-1/X-252-

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L-1/X-415- L-1/X-416- L-1/X-417- L-1/X-418-

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L-2/X-415- L-2/X-416- L-2/X-417- L-2/X-418-

L-3/X-1- L-3/X-2- L-3/X-3- L-3/X-4- L-3/X-5- L-3/X-6-
L-3/X-7- L-3/X-8- L-3/X-9- L-3/X-10- L-3/X-11- L-3/X-12-
L-3/X-13- L-3/X-14- L-3/X-15- L-3/X-16- L-3/X-17- L-3/X-18-
L-3/X-19- L-3/X-20- L-3/X-21- L-3/X-22- L-3/X-23- L-3/X-24-
L-3/X-25- L-3/X-26- L-3/X-27- L-3/X-28- L-3/X-29- L-3/X-30-
L-3/X-31- L-3/X-32- L-3/X-33- L-3/X-34- L-3/X-35- L-3/X-36-
L-3/X-37- L-3/X-38- L-3/X-39- L-3/X-40- L-3/X-41- L-3/X-42-
L-3/X-43- L-3/X-44- L-3/X-45- L-3/X-46- L-3/X-47- L-3/X-48-

L-3/X-49- L-3/X-50- L-3/X-51- L-3/X-52- L-3/X-53- L-3/X-54-
L-3/X-55- L-3/X-56- L-3/X-57- L-3/X-58- L-3/X-59- L-3/X-60-
L-3/X-61- L-3/X-62- L-3/X-63- L-3/X-64- L-3/X-65- L-3/X-66-
L-3/X-67- L-3/X-68- L-3/X-69- L-3/X-70- L-3/X-71- L-3/X-72-
L-3/X-73- L-3/X-74- L-3/X-75- L-3/X-76- L-3/X-77- L-3/X-78-
L-3/X-79- L-3/X-80- L-3/X-81- L-3/X-82- L-3/X-83- L-3/X-84-
L-3/X-85- L-3/X-86- L-3/X-87- L-3/X-88- L-3/X-89- L-3/X-90-
L-3/X-91- L-3/X-92- L-3/X-93- L-3/X-94- L-3/X-95- L-3/X-96-
L-3/X-97- L-3/X-98- L-3/X-99- L-3/X-100- L-3/X-101- L-3/X-102-
L-3/X-103- L-3/X-104- L-3/X-105- L-3/X-106- L-3/X-107- L-3/X-108-
L-3/X-109- L-3/X-110- L-3/X-111- L-3/X-112- L-3/X-113- L-3/X-114-
L-3/X-115- L-3/X-116- L-3/X-117- L-3/X-118- L-3/X-119- L-3/X-120-
L-3/X-121- L-3/X-122- L-3/X-123- L-3/X-124- L-3/X-125- L-3/X-126-
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L-3/X-133- L-3/X-134- L-3/X-135- L-3/X-136- L-3/X-137- L-3/X-138-
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L-3/X-151- L-3/X-152- L-3/X-153- L-3/X-154- L-3/X-155- L-3/X-156-
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L-3/X-169- L-3/X-170- L-3/X-171- L-3/X-172- L-3/X-173- L-3/X-174-
L-3/X-175- L-3/X-176- L-3/X-177- L-3/X-178- L-3/X-179- L-3/X-180-
L-3/X-181- L-3/X-182- L-3/X-183- L-3/X-184- L-3/X-185- L-3/X-186-
L-3/X-187- L-3/X-188- L-3/X-189- L-3/X-190- L-3/X-191- L-3/X-192-
L-3/X-193- L-3/X-194- L-3/X-195- L-3/X-196- L-3/X-197- L-3/X-198-
L-3/X-199- L-3/X-200- L-3/X-201- L-3/X-202- L-3/X-203- L-3/X-204-
L-3/X-205- L-3/X-206- L-3/X-207- L-3/X-208- L-3/X-209- L-3/X-210-

L-3/X-211- L-3/X-212- L-3/X-213- L-3/X-214- L-3/X-215- L-3/X-216-
L-3/X-217- L-3/X-218- L-3/X-219- L-3/X-220- L-3/X-221- L-3/X-222-
L-3/X-223- L-3/X-224- L-3/X-225- L-3/X-226- L-3/X-227- L-3/X-228-
L-3/X-229- L-3/X-230- L-3/X-231- L-3/X-232- L-3/X-233- L-3/X-234-
L-3/X-235- L-3/X-236- L-3/X-237- L-3/X-238- L-3/X-239- L-3/X-240-
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L-3/X-247- L-3/X-248- L-3/X-249- L-3/X-250- L-3/X-251- L-3/X-252-
L-3/X-253- L-3/X-254- L-3/X-255- L-3/X-256- L-3/X-257- L-3/X-258-
L-3/X-259- L-3/X-260- L-3/X-261- L-3/X-262- L-3/X-263- L-3/X-264-
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L-3/X-271- L-3/X-272- L-3/X-273- L-3/X-274- L-3/X-275- L-3/X-276-
L-3/X-277- L-3/X-278- L-3/X-279- L-3/X-280- L-3/X-281- L-3/X-282-
L-3/X-283- L-3/X-284- L-3/X-285- L-3/X-286- L-3/X-287- L-3/X-288-
L-3/X-289- L-3/X-290- L-3/X-291- L-3/X-292- L-3/X-293- L-3/X-294-
L-3/X-295- L-3/X-296- L-3/X-297- L-3/X-298- L-3/X-299- L-3/X-300-
L-3/X-301- L-3/X-302- L-3/X-303- L-3/X-304- L-3/X-305- L-3/X-306-
L-3/X-307- L-3/X-308- L-3/X-309- L-3/X-310- L-3/X-311- L-3/X-312-
L-3/X-313- L-3/X-314- L-3/X-315- L-3/X-316- L-3/X-317- L-3/X-318-
L-3/X-319- L-3/X-320- L-3/X-321- L-3/X-322- L-3/X-323- L-3/X-324-
L-3/X-325- L-3/X-326- L-3/X-327- L-3/X-328- L-3/X-329- L-3/X-330-
L-3/X-331- L-3/X-332- L-3/X-333- L-3/X-334- L-3/X-335- L-3/X-336-
L-3/X-337- L-3/X-338- L-3/X-339- L-3/X-340- L-3/X-341- L-3/X-342-
L-3/X-343- L-3/X-344- L-3/X-345- L-3/X-346- L-3/X-347- L-3/X-348-
L-3/X-349- L-3/X-350- L-3/X-351- L-3/X-352- L-3/X-353- L-3/X-354-
L-3/X-355- L-3/X-356- L-3/X-357- L-3/X-358- L-3/X-359- L-3/X-360-
L-3/X-361- L-3/X-362- L-3/X-363- L-3/X-364- L-3/X-365- L-3/X-366-
L-3/X-367- L-3/X-368- L-3/X-369- L-3/X-370- L-3/X-371- L-3/X-372-

L-3/X-373- L-3/X-374- L-3/X-375- L-3/X-376- L-3/X-377- L-3/X-378-
L-3/X-379- L-3/X-380- L-3/X-381- L-3/X-382- L-3/X-383- L-3/X-384-
L-3/X-385- L-3/X-386- L-3/X-387- L-3/X-388- L-3/X-389- L-3/X-390-
L-3/X-391- L-3/X-392- L-3/X-393- L-3/X-394- L-3/X-395- L-3/X-396-
L-3/X-397- L-3/X-398- L-3/X-399- L-3/X-400- L-3/X-401- L-3/X-402-
L-3/X-403- L-3/X-404- L-3/X-405- L-3/X-406- L-3/X-407- L-3/X-408-
L-3/X-409- L-3/X-410- L-3/X-411- L-3/X-412- L-3/X-413- L-3/X-414-
L-3/X-415- L-3/X-416- L-3/X-417- L-3/X-418-

and so on.

SYNTHESES OF BIVALENT COMPOUNDS

The preferred compounds of Formula I are bivalent. Accordingly, for the purpose of simplicity, the figures and reaction schemes below focus on illustrating the synthesis of bivalent polyene macrolide antibiotics. It should be noted, however, that the same techniques can be used to generate higher order multibinding compounds, i.e., the compounds of the invention where n is 3-10, and one example of synthesis of a trivalent construct is provided.

To further simplify the reaction schemes, only the portion of the ligand that is involved in the reaction is shown (Figures 7 - 9) . In Figures 10 - 17, the ligand is depicted as a triangle with the [O], [N], and [C] functional groups attached to it. Generic primary or secondary amines are shown as R-NHR^y or R-NHR^z, where R^y and R^z can represent either an alkyl group or a hydrogen.

In each of the general descriptions below, reactions are carried out in the dark under an inert atmosphere, e.g. Ar or N_2 gas, due to the sensitivity of the antibiotic ligands. Reactions performed under standard amide coupling conditions

are carried out in an inert polar solvent (e.g. DMF, DMA) in the presence of a hindered base (e.g. TEA, DIPEA) and standard amide coupling reagents (e.g. PyBOP, HATU).

EXAMPLE 1

Hemiketal-linked [O-O] Compounds:

Compounds of Formula I linked via their hemiketal groups, i.e. where the linkage is from the [O] group of a first ligand to the [O] group of a second ligand, may be prepared as shown in Figure 7.

The starting material is a compound of formula (2) having an Fmoc-protected amine group and an allyl ester-protected carboxy group. This compound is synthesized as described by Taylor et. al. (*J. Antibiot. 46: 486-93 (1993)*). Two molar equivalents of the compound of formula (2) are reacted with a molar equivalent of a suitable diol linker (3) in dry tetrahydrofuran in the presence of camphorsulfonic acid to yield the di-Fmoc, di-allyl ester bis(ketal) compound (4). Deprotection of the bis-ketal is carried out sequentially. The Fmoc groups are removed with piperidine in DMSO/MeOH at room temperature. The crude product (5) is collected by precipitation in nonpolar solvent, redissolved in tetrahydrofuran, and treated with pyrrolidine and tetrakis(triphenylphosphine)palladium. The crude product is collected by centrifugation, and further purified by reprecipitation to yield the bis-ketal compound of Formula I (6).

EXAMPLE 2

Carboxy-linked [C-C] Compounds:

Compounds of Formula I linked via their carboxy groups, i.e. where the linkage is from the [C] group of a first ligand to the [C] group of a second ligand, may be prepared as shown in Figure 8.

Two molar equivalents of ligand (**1**) are reacted with one molar equivalent of a diamine linker (**7**) under standard amide coupling conditions. When the reaction is substantially complete, the compound of Formula I (**8**) is isolated, preferably by crystallization from a nonpolar solvent, and is further purified by conventional means, preferably by recrystallization and chromatography.

This coupling is preferably carried out without protecting the amine group on the ligand, which has reduced nucleophilicity due to both electronic and steric effects; however, amine-protecting groups are well known in the art and can be used if desired.

EXAMPLE 3

Amine-Linked [N-N] Compounds:

Compounds of Formula I linked via their amine groups, i.e. where the linkage is from the [N] group of a first ligand to the [N] group of a second ligand, may be prepared as shown in Figure 9.

Two molar equivalents of ligand (**1**) are reacted with one molar equivalent of an N,N'- bis-maleimide linker (**9**) at room temperature for about 1 day in an inert polar solvent, (e.g. DMF) preferably in the presence of a hindered base, (e.g. TEA). This Michael-type addition reaction is described by Czerwinski et al *(J. Antibiot. 44: 979-984 (1991))*. The product of Formula I (**10**) is isolated by

crystallization from a nonpolar solvent and is further purified, preferably by crystallization and chromatography.

Alternatively, the compound of Formula I (**12**) may be prepared by acylation with a dicarboxylic linker. One molar equivalent of dicarboxylic linker is dissolved in an inert polar solvent, and activated with a standard amide coupling reagent (e.g., DCC, DPPA, HBTU) to form activated dicarboxylic linker (**11**). Two molar equivalents of ligand (**1**) are then reacted with the activated linker (**11**) under standard amide coupling conditions. When the reaction is substantially complete, the compound of Formula I (**12**) is isolated, preferably by crystallization from a nonpolar solvent, and is further purified by conventional means, preferably by recrystallization and chromatography.

[N-C] Linked Compounds:

Compounds of Formula I linked head-to-tail via the carboxyl group [C]on one ligand and the amine group [N] of a second ligand may be prepared by sequential coupling to a linker bearing appropriate complementary functional groups. For example, ligand (1), optionally modified as an amide or ester derivative on the [C] terminus, is acylated on the amino group [N] with an Fmoc- protected amino acid in a reaction mediated by standard coupling reagents. (PyBOP/HOBT or HATU/HOAT) in an inert polar solvent (e.g., DMF) at room temperature for about 1 day; potentially in the presence of a hindered base (e.g., DIPEA). The N-acylated product is isolated by precipitation from a nonpolar solvent and is further purified, preferably by chromatography. The Fmoc protecting group on the linker is subsequently removed preferably using an amine base (e.g., piperidine) in a polar solvent (e.g., DMF). The product is isolated by precipitation from a non-polar solvent and is purified, preferably by chromatography. This compound is the coupled

with another ligand (l) which is optionally protected or otherwise modified at the [N] terminus. The coupling is mediated by standard coupling reagents (e.g., PyBOP/HOBT) in an inert polar solvent (e.g., DMF) preferably in the presence of a hindered base (e.g., DIPEA) for about 1 day at about room temperature. The product is isolated by precipitation from an apolar solvent and purified preferably by chromatography. If protecting groups are present, they are subsequently removed under standard conditions.

Alternatively, [N-C] dimers may be prepared by initial [N] alkylation of liand (l) (e.g., by relative alkylation with a protected amino aldehyde) followed by coupling to the [C] terminus of a second ligand (l).

Compounds of Formula I where p=3-10

The strategies described above can be used for preparing higher-order valency compounds of Formula I, i.e., compounds with p=3-10. As shown in Figure 10, compounds (**14**) and (**16**) are prepared by coupling ligand (**1**) to a central core bearing multiple functional groups. The reaction conditions are the same as described above for the preparation of bivalent compounds, with appropriate adjustments made in the molar quantities of ligand and reagents.

Another strategy for making higher-order valency compounds of Formula I which can be applied to all ligands, is to introduce a 'spacer' before coupling to a central core. Such a spacer can itself be chosen from the possible core compounds, and comprises a heterobifunctional spacer. The ligand is coupled to the spacer, and the resulting product is then coupled, after deprotection if necessary, with an appropriate central core. Examples of this type of linking are shown in Figures 11 and 12. The spacer is represented in these figures by a gray circle.

Referring to Figure 11, which illustrates the preparation of a [C-C] bivalent compound of Formula I, the ligand (**1**) is reacted with about 1.1 molar equivalents of a heterobifunctional compound of formula (**21**), where R^y and R^z are independently hydrogen or alkyl, under conventional amide coupling conditions. When the reaction is substantially complete, a compound of formula (**22**) is isolated and purified by conventional means.

The carbamic ester moiety is chosen for ease of removal under mild conditions in subsequent reactions, and is preferably 9-fluorenylmethoxycarbonyl (Fmoc). A compound of formula (**22**) is reacted with a mild base (preferably piperidine) to remove the protecting group, which also affords decarboxylation. The reaction is conducted in an inert polar solvent (preferably DMF) at about room temperature for about 10 minutes to one hour. When the reaction is substantially complete, a compound of formula (**23**) is isolated and purified by conventional means, preferably crystallization and chromatography.

A compound of formula (**23**) is then converted into a [C-C] bivalent compound by reaction with a dicarboxylic acid. In general, about 3 molar equivalents of a compound of formula (**23**) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula (**11**), under conventional amide coupling conditions. When the reaction is substantially complete, a compound of Formula I (**24**) is isolated and purified by conventional means, preferably crystallization and chromatography.

Figure 12 illustrates the preparation of a [N-N] bivalent compound of Formula I. The starting material, a "carboxy-capped" ligand (**25**) is prepared by reacting approximately 1 molar equivalent of ligand with an appropriately

substituted amine, for example, an alkyl amine, under standard amide coupling conditions. After several days, or when the reaction is substantially complete, the "carboxy-capped" adduct is isolated by precipitation in a nonpolar solvent, and is further purified by reprecipitation and chromatography, for example, on silica gel.

The "carboxy-capped" ligand (**25**) is reacted with about 1.1 molar equivalents of a heterobifunctional compound of formula (**26**) under conventional amide coupling conditions. When the reaction is substantially complete, a compound of formula (**27**) is isolated and purified by conventional means.

This compound (**27**) is deprotected with a mild base (preferably piperidine) in an inert polar solvent (preferably DMF) at room temperature for about 10 minutes to one hour. When the reaction is substantially complete, a compound of formula (**28**) is isolated and purified by conventional means, preferably by crystallization and chromatography.

About 3 molar equivalents of the compound of formula (**28**) is reacted with about 1 molar equivalent of a dicarboxylic acid of formula (**11**) under conventional amide coupling conditions. When the reaction is substantially complete, a compound of Formula I (**29**) is isolated and purified by conventional means, preferably by crystallizatioon and chromatography.

In its simplest form, where the grey circle represents a methylene group (with or without sidechain), this strategy is equivalent to aminoacylation, and is well known from polypeptide synthesis.

All of the synthetic strategies described above employ a step in which the ligand, attached to spacers or not, is symmetrically linked to a central core in a single reaction to give a bivalent or higher-order valency multibinding compound. Compounds of Formula I can also be synthesized using an asymmetric, linear approach This strategy is preferred when linking two or more ligands at different points of connectivity (e.g., for the preparation of multibinding compounds linked [C-N]).

In the first example, a compound of formula (**28**), prepared as shown in Figure 13, is coupled with an equimolar amount of "amine-capped" ligand (**30**), using conventional amide coupling conditions, to form a bivalent compound of formula I (**31**). The "amine-capped" ligand (**30**) is prepared by reacting approximately 1 molar equivalent of ligand with an appropriate acylating agent, such as an acyl halide, in an inert organic solvent (e.g., DMF) in the presence of a hindered base, preferably triethylamine.

A method of preparing multibinding compounds where n is 3-10 is illustrated in Figure 14. In this example, the ligands are linked in a discontinuous chain, represented as L-(X-L-X)$_{p-2}$ -L.

Referring to Figure 14, an Fmoc-protected ligand (**32**) is substituted for (**30**) in reaction scheme of Figure 13. After coupling, the product is deprotected to reveal an amine for further linking. This compound is then coupled to another compound of formula (**26**), and the cycle is repeated until the desired valency is reached. The final step is the addition of an "amino capped" ligand (**30**) to yield a compound of formula (**33**), which is isolated and purified as previously described.

A linear strategy can also be extended to the synthesis of heterovalomers, examples of which are shown in Figure 15.

Utility and Testing

The multibinding compounds of this invention are useful for their antifungal and antiprotozoal activity. They will be typically be used for the treatment of diseases and conditions in mammals that involve or are mediated by pathogenic fungi, yeasts and protozoans, and are particularly important for use in immunocompromised patients with opportunistic systemic fungal and yeast infections. These compounds are expected to possess immunostimulatory activity and may be used alone or in combination with cytokine and/or cytokine antagonists to enhance resistance to, and treat infections of, fungi, bacteria and parasites in immunocompromised hosts (*see, e.g., Hartsel and Bolard, Trends Pharmacol. Sci. 17: 445 (1996);Cenci et al, J. Infectious Dis. 176: 217 (1997)).*

The multibinding agents of this invention are also expected to be useful in the prophylaxis and/or treatment of diseases caused by lipid-enveloped viruses and as potentiators of antiviral drugs (*see, e.g., Pleskoff et al, J. Virol 69: 570 (1995); Aloia et al, Proc. Natl. Acad. Sci. U.S.A. 90: 5181 (1993); Malewicz et al, Antimicrob. Agents and Chemotherapy 25: 772 (1984)*).

As well, these agents may be useful for controlling infections of agricultural crops, e.g., those caused by *Fusarium spp. and Aspergillus flavus.*

The compounds of Formula I are tested for activity as described below in Examples 9-12. Methods for assessing resistance to yeast infection and immunomodulatory effects on T cell immunity are performed as described in

Cenci et al, J. Infect. Dis. 176: 217 (1997). Potentiation of antiviral activity is carried out according to *Malewicz et al, Antimicrob. Agents Chemother. 25: 772 (1984).* Antibacterial activity is assayed in vitro by measuring minimal inhibitory concentrations (MIC_{50}, MIC_{90}) and minimum bactericidal concentrations (MBC) standard microdilution procedures according to guidelines of the National Committee for Clinical Laboratory Standards. Bactericidal activity is also evaluated by time-kill curve tests according to standard procedures of the National Committee for Clinical Laboratory Standards. These assays are described in *Lorean, "Antibiotics in Laboratory Medicine", 4th Ed.*

Pharmaceutical Formulations and Administration

When employed as a pharmaceutical for human and veterinary uses, the compounds of formula I or their pharmaceutically acceptable salts are usually administered in the form of pharmaceutical compositions. These compositions contain, as the active ingredient, one or more of the compounds of formula I above associated with pharmaceutically acceptable excipients, carriers, diluents, permeation enhancers and adjuvants.

Administration of an appropriate pharmaceutical composition can be carried out via any of the accepted modes of administration of agents having similar utilities. Thus, administration can be parenteral (e.g., intravenous, intraarterial, subcutaneous, intramuscular, intrapleural, intralymphatic, or intraperitoneal), internal or topical, as may be required by the condition to be treated and/or the pharmacokinetics desired. The compounds of formula I may be administered alone or in combination with other known antifungal agents, chemotherapeutic agents or surfactants.

The compositions are prepared in a manner well known in the pharmaceutical art (*see, e.g., Remington's Pharmaceutical Sciences,* Mace Publishing Company, Philadelphia, PA, 17th ed. (1985) and "*Modern Pharmaceutics*", Marcel Dekker, Inc., 3rd ed. (G.S.Banker & C.T. Rhodes, eds.). They can be formulated as tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders. Pharmaceutically acceptable salts of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, e.g., by *J. March, Advanced Organic Chemistry: Reactions, Mechanisms and Structure, 4th Ed. (New York: Wiley-Interscience, 1992).*

Preferred formulations for treating disseminated fungal infections with compounds of formula I are those wherein the active ingredient is complexed with lipids or incorporated into liposomes. Examples of useful formulations are given in U.S. Patent Nos. 5,032,582, 5,616,334, 5,043,107 and 4,812,312. Stealth liposomes and liposomes adapted for targetting to particular tissues are also expected to be useful for practicing this invention. Other preferred formulations are those that are expected to improve the water solubility of poorly soluble compounds of formula I. For example, such compounds can be encapsulated with cyclodextrins, such as hydroxyalkylated gamma cyclodextrin (see, e.g., PCT Application No. WO 89/10739), conjugated with polysaccharides (US Patent No. 5,567,685), oligo(ethylene glycol) (U.S. Patent No. 5,606,038), and derivatized with guanidine salts and esters (U.S. Patent No. 4,396,610).

Generally, depending on the intended mode of administration, the compound of formula I above is employed at no more than about 10 weight percent of the pharmaceutical composition, more preferably between about 1-5 weight percent, with the balance being pharmaceutically inert excipients. Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.5 to about 10 mg, more usually about 1 to about 5 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. For disseminated fungal infections, the preferred dosage of amphotericin B administered intravenously is from about 1 to about 10mg/kg body weight/day, more preferably about 2 to about 5 mg/kg body weight/day.

The compounds of the present invention, or their pharmaceutically acceptable salts, are administered in a therapeutically effective amount. It will be understood, however, that the amount of the compound of formula I actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

EXAMPLES

The following examples are used to specifically illustrate the attachment of several classes of ligands to linkers according to this invention. The specific ligands employed are for illustrative purposes and should not be construed as a limitation for this application.

EXAMPLE 1

Ethylene Glycol Linked Bivalent [O-O] Compounds of Formula I

Referring to the synthesis shown in Figure 7, a solution of *N*-(9-fluorenylmethoxycarbonyl)amphotericin B allyl ester (2.16 mmol, 2.51 g, prepared as described by Taylor et al. *J. Antibiot.* **1993**, *46*, 486-493) and ethylene glycol (1.1 mmol, 0.067 g) in 25 mL dry THF is cooled in an ice bath and treated with camphorsulfonic (0.58 mmol, 0.145 g). After stirring for 30 minutes, ethyl acetate and sodium bicarbonate solution are added. The resulting organic phase is isolated, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product is chromatographed on silica gel using 10:1 methylene chloride:methanol eluent to afford the di-Fmoc, di-allyl ester bis(ketal). The Fmoc groups of the bis(ketal) are then removed at room

temperature using a two-fold excess of piperidine in DMSO:methanol solvent. The crude product is precipitated from the reaction mixture by pouring it into diethyl ether. After drying *in vacuo*, the allyl esters are removed at room temperature using a combination of tetrakis(triphenylphosphine) palladium[0] and pyrrolidine in THF solvent. The crude product precipitates from the reaction mixture, is collected by centrifugation, washed with THF, and then re-precipitated by dissolving in a 5:1 mixture of methanol:THF and diluting into diethyl ether. Drying *in vacuo* affords 13-0, 13'-0'(1,2-ethandiyl)bis(amphotericin B).

In the same way, substituting alternative diols for ethylene glycol, additional bivalent [O-O] compounds of Formula I are obtained.

EXAMPLE 2

Bivalent [C-C] Compounds of Formula I having a linker of Formula -$NHCH_2(CH_2OCH_2)_3 CH_2NH$-

Referring to the synthesis shown in Figure 8, amphotericin B (0.0554 g, 0.060 mmol) is suspended in 2 mL of DMF, stirred at room temperature, and then treated with triethylamine (0.60 mmol), PyBOP (0.016 g, 0.060 mmol), and 1,11-diamino-3,6,9-trioxaundecane (0.0058 g, 0.030 mmol) in the dark under a nitrogen atmosphere. After 5 days, the reaction mixture is poured into 80 mL of diethyl ether. The crude product is further purified, first by four times resolubilizing in methanol and reprecipitating from diethyl ether, and then by silica gel chromatography using 9:1 methanol:30% ammonium hydroxide eluent to afford bis(amphotericin B)-3,6,9-trioxaundecan-1 ,1 1-diamide.

EXAMPLE 3

Bivalent [C-C] Compounds of Formula I having a linker of Formula -$NH(CH_2)_8NH$-

Fmoc Amphotericin B (1.15 g, 1.00 mmol, prepared as described by Driver et al. *Tetrahedron Lett.* **1992**, *33*, 4357-4360) is dissolved in 10 mL anhydrous dimethylformamide, stirred at room temperature, and treated sequentially with diisopropylethylamine (348 μmL, 2.00 mmol), 1,8-diaminooctane (0.072 g , 0.50 mmol), HOBT (0.135 g,1.00 mmol), and PyBOP (0.520 g, 1.00 mmol). The resulting mixture is stirred for 24 h and then added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methylene chloride:methanol eluent to provide the di-Fmoc protected [C-C] compound of Formula I having a linker of formula -$NH(CH_2)_8NH$-.

The di-Fmoc compound (0.480 g, 0.200 mmol) is dissolved in 5 mL anhydrous dimethylformamide and treated with excess piperidine (250 μL). After stirring two hours at room temperature, the reaction mixture is diluted with 5 mL methanol and added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methanol:30% aqueous ammonia eluent to provide bis(amphotericin B)-1,8-octanediamide.

Using either the methods described in Examples 2 or 3, substituting alternative diamines, additional bivalent [C-C] compounds of Formula I are obtained.

EXAMPLE 4

Bis-maleimide Linked Bivalent [N-N] Compounds of Formula I

Referring to the first synthesis shown in Figure 9, to a stirred suspension of amphotericin B (0.277 g, 0.30 mmol) in 5 mL DMF is added triethylamine (0.042 mL, 0.300 mmol) and then *N,N'*-1,6-hexandiyl-bis(maleimide) (0.041 g, 0.15 mmol). The mixture is stirred at room temperature for 1 day. The crude product is precipitated by the addition of 250 mL diethyl ether, washed with additional ether, and dried *in vacuo*. This material is further purified by silica gel chromatography using 13:6:1 chloroform:methanol:water eluent to afford bis(amphotericin B)-*N,N'*-1,6-hexandiyl-bis(succinamide).

In the same way, substituting alternative bis(maleimide)s for *N,N'*-1,6-hexandiyl-bis(maleimide), additional bivalent [N-N] compounds of Formula I are obtained.

EXAMPLE 5

Bivalent [N-N] Compounds of Formula I having a Linker of Formula -C(O)(C_6H_4)C(O)-

Referring to the second synthesis shown in Figure 9, amphotericin B (0.92 g, 1.0 mmol) is slurried in 50 mL anhydrous dimethylformamide, stirred at room temperature, and treated sequentially with terephthalic acid (0.083 g, 0.50 mmol), diisopropylethylamine (350μL, 2.0 mmol), HOBT (0.14 g, 1.0 mmol), and PyBOP (0.52 g, 1.0 mmol). After 24 hours the mixture is added dropwise with vigorous stirring to 1 L anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methylene chloride:methanol eluent to provide bis(amphotericin B)-terephthalamide.

In the same way, substituting alternative diacids for terephthalic acid, additional bivalent [N-N] compounds of Formula I are obtained.

EXAMPLE 6

Bivalent [N-N] Compounds of Formula I having a Linker of Formula $-(CH_2)_2NHC(O)(CH_2)_2C(O)NH(CH_2)_2-$

N-[(2-aminoethyl)-Amphotericin B, allyl ester (0.101 g, 0.100 mmol, prepared as described in Example 16 (below) is dissolved in 5 mL anhydrous dimethylformamide, stirred at room temperature, and treated sequentially with succinic acid (0.0059 g, 0.050 mmol), diisopropylethylamine (17μL, 0.10 mmol), HOBT (0.014 g, 0.10 mmol), and PyBOP (0.052 g, 0.10 mmol). After 24 hours the mixture is added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methylene chloride:methanol eluent to provide a protected bivalent [N-N] compound of Formula I as the di-allyl ether.

The diallyl ether (0.079 g, 0.040 mmol) is dissolved in 10 mL anhydrous tetrahydrofuran, stirred under nitrogen at room temperature, and treated sequentially with pyrrolidine (11 μL, 0.12 mmol) and tetrakis(triphenylphosphine)palladium[0] (0.010 g, 0.010 mmol). After 1 hour, the precipitate is collected by centrifugation, washed with tetrahydrofuran, and dried in vacuo to afford the title bivalent [N-N] compound of Formula I.

In the same way, substituting alternative diacids for succinic acid, and alternatives to *N*-[(2-aminoethyl)-Amphotericin B, allyl ester, additional bivalent [N-N] compounds of Formula I are obtained.

EXAMPLE 7

Bivalent [N-C] Compounds of Formula I having a Linker of Formula -$NH(CH_2)_2$-

Referring to the synthesis shown in Figure 16, Fmoc Amphotericin B, allyl ester (1.19 g, 1.00 mmol, prepared as described by Taylor et al. *J. Antibiot.* **1993**, *46*, 486-493) is dissolved in 10 mL anhydrous dimethylformamide and treated with excess piperidine (500 µL). After stirring two hours at room temperature, the reaction mixture is diluted with 10 mL methanol and added dropwise with vigorous stirring to 200 mL anhydrous diethyl ether. The resulting precipitate is redissolved in methanol and precipitated from diethyl ether to afford Amphotericin B, allyl ester which is used without further purification.

Amphotericin B, allyl ester (0.482 g, 0.50 mmol) is dissolved in 10 mL 1:1 anhydrous dimethylformamide:anhydrous methanol and treated sequentially with diisopropylethylamine (87 µL, 0.50 mmol) and Fmoc-glycinal (141 mg, 0.50 mmol). After stirring one hour at room temperature, the reaction mixture is cooled in an ice bath and treated with sodium cyanoborohydride (0.016 g, 0.25 mmol) followed by trifluoroacetic acid (77 µL, 1.00 mmol). The mixture is removed from the ice bath and allowed to warm to room temperature and stir for 2 hours. The reaction mixture is then poured into 100 mL ethyl acetate and extracted with 100 mL sodium bicarbonate solution. The organic extract is dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product is fractionated by chromatography on silica gel using 9:1 methylene chloride:methanol eluent to afford *N*-[(2-fluorenylmethylcarbamato)-Amphotericin B, allyl ester.

N-[(2-fluorenylmethylcarbamato)-Amphotericin B, allyl ester (0.249 g, 0.200 mmol) is dissolved in 5 mL anhydrous dimethylformamide and treated with excess piperidine (250 µL). After stirring two hours at room temperature, the reaction

mixture is diluted with 5 mL methanol and added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methanol:30% aqueous ammonia eluent to afford *N*-[(2-aminoethyl)-Amphotericin B, allyl ester.

N-[(2-aminoethyl)-Amphotericin B, allyl ester (0.101 g, 0.100 mmol) and Fmoc Amphotericin B (0.119 g, 0.100 mmol, prepared as described by Driver et al. *Tetrahedron Lett.* **1992**, *33*, 4357-4360) are dissolved in 5 mL anhydrous dimethylformamide, stirred at room temperature, and treated sequentially with diisopropylethylamine (17 μL, 0.10 mmol), HOBT (0.014 g, 0.10 mmol), and PyBOP (0.052 g, 0.10 mmol). After 24 hours the mixture is added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methylene chloride:methanol eluent to provide a protected bivalent [N-C] compound of Formula I having a linker of formula -$NH(CH_2)_2$-, [Fmoc-Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B, allyl ester].

The protected bivalent [N-C] compound [Fmoc-Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B, allyl ester] (0.11 g, 0.050 mmol) is dissolved in 10 mL anhydrous tetrahydrofuran, stirred under nitrogen at room temperature, and treated sequentially with pyrrolidine (13 μL, 0.15 mmol) and tetrakis(triphenylphosphine)palladium[0] (0.010 g, 0.010 mmol). After 1 hour, the precipitate is collected by centrifugation, washed with tetrahydrofuran, and dried in vacuo. This material, crude [Fmoc-Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B], is then dissolved in 5 mL anhydrous dimethylformamide and treated with excess piperidine (250 μL). After stirring two hours at room temperature, the reaction mixture is diluted with 5 mL methanol and added

dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is collected by filtration and dried in vacuo. The crude product is fractionated by silica gel chromatography using 9:1 methanol:30% aqueous ammonia eluent to afford the bivalent [C-N] compound of Formula I having a linker of formula $-NH(CH_2)_2-$, [Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B].

In the same way, substituting alternative protected amino aldehydes for Fmoc-glycinal in the second step of this procedure, additional bivalent [C-N] compounds of Formula I are obtained.

EXAMPLE 8

Trivalent [N-C-N] compound of Formula I.

Referring to the synthesis as shown in Figure 17, [Fmoc-Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B] (0.10 g, 0.045 mmol, prepared as described in Example 7) and *N*-[(2-aminoethyl)-Amphotericin B, allyl ester (0.045 g, 0.045 mmol, prepared as described in Example 6) are dissolved in 5 mL anhydrous dimethylformamide, stirred at room temperature, and treated sequentially with diisopropylethylamine (8 μL, 0.05 mmol), HOBT (0.07 g, 0.105 mmol), and PyBOP (0.026 g, 0.050 mmol). After 24 hours the mixture is added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is collected by centrifugation and dried in vacuo. The material is dissolved in 10 mL anhydrous tetrahydrofuran, stirred under nitrogen at room temperature, and treated sequentially with pyrrolidine (13 μL, 0.15 mmol) and tetrakis(triphenylphosphine)palladium[0] (0.010 g, 0.010 mmol). After 1 hour, the precipitate is collected by centrifugation, washed with tetrahydrofuran, and dried in vacuo. The material is redissolved in 5 mL anhydrous

dimethylformamide and treated with excess piperidine (250 μL). After stirring two hours at room temperature, the reaction mixture is diluted with 5 mL methanol and added dropwise with vigorous stirring to 100 mL anhydrous diethyl ether. The resulting precipitate is fractionated by silica gel chromatography using 9:1 methanol:30% aqueous ammonia eluent to afford a trivalent [N-C-N] compound of Formula I, Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B]-[(*N*-(2-amidoethyl)-Amphotericin B].

EXAMPLE 9

Assay of Antifungal Activity

Antifungal activity (MIC) of compounds of Formula I is tested against a panel of drug-sensitive and drug-resistant organisms that includes the following species and strains:

Saccharomyces cerevisiae, Candida albicans, Aspergillus (A. niger A. nidulans, A. fumigatus, A. flavus), Cryptococcus neoformans, Coccidioides immitis, Torulopsis glabrata, Histoplasma capsulatum, Fusarium spp. and Blastomyces dermatitidis.

Compounds are diluted two-fold in Sabouraud's liquid medium in wells of a microtiter plate. Cell suspensions are inoculated into wells to provide a final concentration of 10^3 cells per ml. Spore suspensions of *Aspergillis* are inoculated to a final concentration of 10^4 spores per ml. The total volume per well is adjusted to 100 μL. Plates are incubated at 37°C for 2 days and the turbidity of each well is then assessed visually. The minimum inhibitory concentration (MIC) of the multibinding compounds are compared with that of the monovalent ligand, the monovalent ligand conjugated to a linker, and a non-polyene macrolide

monovalent antifungal agent such as fluconazole. The MIC is the lowest concentration of compound that prevents visible growth.

EXAMPLE 10

Leishmanicidal activity

The promastigote of *Leishmania major* is cultured in Medium 199 supplemented with 10% heat inactivated fetal bovine serum (FBS) and 2% urine. The growth of the promastigote is monitored over a period of 5 days in the absence and presence of the compounds of Formula I or controls (see Example 8 above). The EC_{50} value is the concentration of drug necessary to inhibit the growth rate of cells to 50% of the rate in growth medium minus drug.

EXAMPLE 11

In vitro assays for mammalian cell toxicity

A. *Assay of hemolytic activity*

Toxicity of the compounds of Formula I to human erythrocytes is assessed by incubating fresh washed human red blood cells at a final concentration of 3 x 10^7 cells/ml in microtiter plate wells containing two-fold serial dilutions of the compounds. After a 1 hour incubation at 37°C , the turbidity of the cell suspensions is measured at 492nm in a microtiter plate reader. The concentration of the compound that produces a 50% decrease in turbidity of the whole cell suspension is determined as the EH_{50}.

B. *Release of lactic dehydrogenase (LDH)*

HT29 cells (ATCC) are seeded in triplicate at a density of 3 x 10^4 cells per well in 25-well plastic tissue culture plates in RPMI 1640 medium and incubated for 24 hours at 37°C in a 5%CO_2-95% air atmosphere. Compounds of Formula I

are added to the cultures to give final concentrations of 10^{-5}-10^{-8} M and the cultures are incubated for an additional 18 hours. At this time, LDH activity released into the supernatant is measured spectrophotometrically by a NADPH-coupled assay *(Bergmeyer, H.U. et al, (1963) pp. 736-741 In: H.U. Bergmeyer (ed.) Methods of enzymatic analysis. Verlag Chemie, Weinheim, Germany.)* The value for 100% of LDH release is obtained by lysing the cells.

C. *Potassium release from erythrocytes* (EK_{50})

Erythrocytes are suspended in 150mM choline chloride, pH 7.4, buffered with 5mM Tris-Cl, and incubated for 1 hour at 37°C with various amounts of the test or control compounds (described above in Example 8). After 1 hour, the samples are centrifuged and the supernatant potassium concentration is determined by flame photometry. The value for 100% potassium release is obtained by lysing the cells.

EXAMPLE 12

Measurement of in vivo efficacy of compounds of Formula I

A. *Mouse model of disseminated trichosporonosis*

Cyclophosphamide plus prenisolone-immunosuppressed ICR mice are injected intravenously with a lethal inoculum of *Trichosporon beigelii* (6×10^6 CFU/mouse). Twenty-four hours post inoculation and for 7 days afterwards, the mice are divided into three treatment groups. Each group is dosed once a day intraperitoneally with an amphotericin compound of Formula I at a dose of 0.5 or 2 mg/kg/da , or fluconazole (10 or 40 mg/kg/day) or amphotericin B (0.5 or 2 mg/kg/day). The daily survival rates are measured for each group and for no-treatment controls. The assay is described in detail in Chemotherapy 44: 55-62 (1998).

B. *Rabbit model of experimental Aspergillus fumigatus endocarditis*

Rabbits are treated intravenously with two doses of each compound to be tested at 1.5 mg/kg each at 4 hours and at 30 minutes prior to challenge with an inoculum of *A.. fumigatus*. The animals are sacrificed after three days and the concentration of fungi is measured in the aorta. The assay is described in detail in Mayo Clinic Proceedings 72: 1022-1027 (1997).

C. *Assay for activity against Candida and Cryptococcus*

C. albicans CBS 562 is injected as a 0.1 ml solution into the tail vein of male albino BALB/c mice (approximately 20 grams weight). Inocula of 5×10^4 to 10^5 yeast cells per mouse typically produce systemic infections with no survivors within 10-15 days. Twenty-four hours after infection, mice are treated once daily for 5 days either with a nystatin-containing compound of Formula I, or control compounds as described above in Example 9, or solvent alone at the maximum tolerated dose(i.e., a dose equivalent to approximately 1 mg/kg nystatin in 2% DMSO). The number of survivors in each group is recorded daily over a period of 45 days.

Similar assay conditions are used for *Cryptococcus*-infected mice.

While the present invention has been described with reference to specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the

present invention. All such modifications are intended to be within the scope of the claims appended hereto.

All of the publications, patent applications and patents cited in this application are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

Table 1

Classification of Polyene Macrolide Antibiotics

Number of ring atoms	Class	Antibiotics
20	Tetraene	Aureofuscin
24	Pentaene	Rectilavendomycin
26	Tetraene	Arenomycin B, lucensomycin, pimaricin, tetramycins A and B, tetrins A and B
	Pentaene	Aurenin
28	Tetraene	Rimocidin
	Pentaene	Fungichromin, elizabethin, chainin, filipins I, II, III, and IV
30	Pentaene	Eurocidins A and B
31 or 33 (?)	Pentaene	Gannibamycin
32	Oxo-pentaene	Mycoticins A and B
35	Triene	Rapamycin, demethoxyrapamycin
36	Oxo-pentaene	Roflamycoin
	Oxo-hexaene	Dermostatins A and B
38	Tetraene	Nystatins A_1 and A_3, polifungin B
	Heptaene	Amphotericin B, aureofungin A, candidin, hamycin, levorin A_2, mycoheptin, partricins A and B, perimycin A, DJ-400-B_1 and B_2, 67-121 A and C
44	Pentaene	Lienomycin

(Taken from Ōmura and Tanaka, Chapter 9 in: Macrolide Antibiotics (1989) Academic Press.)

Table 2
Polyene Macrolide Antibiotic Ligands and Uses

Name	Reference	Uses	Figure
A-2	1		
A-7	1		
A-288a	1		
A-288b	1		
A-435	1		
A-5283	1		
Abkhazomycin	1		
AB021-a	24	antifungal (plants)	
AB021-b	24	antifugal (plants)	
AB-161-2	1		
Acmycin	3		
AE-56	1		
AF-1231	1		
AK-15	25	antifungal, antiyeast, antibacterial (gram positive)	
Akitamycin	1		
Aliomycin	1		
Amphotericin A	1	antifungal	
Amphotericin B	1 & 4	antifungal, potentiates acyclovir	2
Antifongine	1		
Arenomycin B	1		10
Ascosin	1	antifungal	
Aurantinin	1	antibacterial	
Aureofacin	5	antimycotic (animals)	
Aureofungin A	1	antifungal	15
Aureofungin B	42	antifungal	
Aureofusin	1		
Aurenin	1		4
Azacolutin (F-17-C)	1	antifungal	
Bannibamycin			
Barodamycin	1		
B-34	1		
BH-890 a	1		
BH-890 b	1		
BK-217 b	1		
BK-217 g	1		
BL-617	1		
Candicidin	1	antimycotic (animals)	
Candicidin D	26	antifungal	18
Candidin	1		3
Candidoin	1		
Candihexin A	1		
Candihexin B	1		
Candimycin	1		
Capsicidin	1		
Chainin	1		17
Chromin	1		
Chromotriemin	1	antifungal	

Cogomycin	7		17
Cryptocidin	1		
Cryptomycin	1		
Demethoxyrapamycin	1	antifungal	11
Durhamycin	1		
DJ-400-B1	1		16
DJ-400-B2	1		16
12-Deoxyroflamycoin		antifungal	
Dermastatin A (polyacetonide)	1	antiviral	14
Dermastatin B	1	antiviral	14
Elizabethin	1		17
Enamine	8	antimycotic	
Endomycin A	1		
Endomycin B	1		
Eurocidin A	1		6
Eurocidin B	1		6
Eurotin A	1		
Faeriefungin	11	antifungal, antibacterial (gram-positive, antiprotozoal)	
Filipin I	1		17
Filipin II	1		17
Filipin III	1		17
Filipin IV	1		17
Flavacid	1		
Flavofungin	34	antifungal	
Flavomycoin	9	antifungal, antibacterial	
Flavumycin A	1		
Flavumycin B	1		
Flavoviridomycin	1		
Fulvomycin A	1		
Fulvomycin B	1		
Fulvomycin C	1		
Fulvomycin O	1		
Fungichromatin	1		
Fungichromin	1		17
Fumanomycin	1		
G 8	1		
G-83	1		
Gangtokmycin	1		
Gannibamycin	1		1
Gedamycin	27	antifungal, hemolytic	
Genimycin	1		
Grecomycin	1		
Griseocarnin	1		
Grubilin	1		
Guamycin			
3874 H1-6(I)	2	antimycotic	
HA-106	1		
HA-135	1		
HA-145	1		

HA-176	1		
Hamycin	41	hypocholesterolic activity, prostate hyperplasia inhibitor	18
Hamycin X	1		
Haptafungin A	1		
Hepcin	1		
Heptamycin	1		
Hexaene H-85		antimycotic	
Hexafungin	1		
Hexamycin	1		
Hexin	1		
HM17	12	antifungal (plant, animal)	
Homochainin	1		
Hydroheptin	1	antifungal	
J4-B	1		
JA-20	13	antifungal, lipid metabolism, and prostate activity	
Kabicidin	1		
Kokandomycin	1		
LIA-0735	1		
Lagosin	28	antimycotic (animals)	17
Levorin A0	1		
Levorin A1	1		
Levorin A2	1	antimycotic, antibacterial	18
Levorin A3	1		
Levorin A4	1		
Levorin B	1		
LIA-0677	1		
LIA-0735	1		
Lienomycin	1	antitumor	7
Lymphosarcin	1		
Lucensomycin	1		10
Lucknomycin	1		
Macrolactin A	29		
Mediocidin	1		
Meparticin	14	antimycotic (animals)	
Moldcidin A	1		
Monicamycin	1		
MS-8209	30	anti-scrapie	
MYC-4	1		
Mycelin IMO	1		
Mycoheptin A1	1		3
Mycoheptin A2	1		
Mycopentin-I	1		
Mycoticin A	31	antifungal	8
Mycotilcin B	31	antifungal	8
Mycotrienin	1	antifungal	
Natamycin	1	antimycotic (animals)	
Neopentaene	1		
Nigericin			
Norchainin	1		

Nystatin A1	1	antimycotic (animals)	
Nystatin A2	1		
Nystatin A3	1		
O-185-I	1		
Onomycin-1	1		
PA-5	15	antifungal, antiyeast	
PA-7	15	antifungal, antiyeast	
PA-86	1		
PA-150	1		
PA-153	1		
PA-166	1		
Particin A	1		18
Particin B	1		18
Pentacidin	1		
Pentafungin	1		
Pentamycin	1		
Perimycin	16		
Perimycin A			9
Pimaricin	17	antifungal, food preservative	10
Pimarolide	32		
Plumbomycin B	1		
Polifungin B	1		
Protomycinolide IV	33		
Protocidin	1	antifungal	
Proticin	1	antibacterial	
Quinquamycin	1		
R-42-B	1		
Rapamycin	1	antifungal	11
Rectiluvendomycin	1		
Resistaphylin	1	antibacterial	
Rimocidin	1		12
RK397	19	tumors	
Robigocidin A	1	antifungal	
Roflamycoin	1	antimycotic	13
Roseofungin	1	antifungal, antiviral (animal)	
Roxaticin	18		
S-728	1		
S-113	21	agricultural mycoses	
T-2636 M	1		
Takamycin (C-11)	1		
Tolimycin	1		
Tetrapol A159	35	antimycotic (plants)	
Tetrafibricin	20	fibrinogen receptor antagon-	
Tetrafungin	1		
Tetramedin	1		
Tetramycin A	1	antifungal	10
Tetramycin B	1	antifungal	10
Tetramycoin A	1		
Tetramycoin B	1		
Tetrin A	1		10
Tetrin B	1		10

Toyamycin	1		
Trichomycin A	1	antifungal, antiyeast	
Trichomycin B	1	antifungal, antiyeast	
Trienine	1	antitumor	
Unamycin	1		
Vacidin A	22	antifungal, induces ion-selective ion channels in rbcs	18
Viridenomycin		antimycotic, antibacterial	
Xantholicin A	1		
Xantholicin B	1		
YS-822A	23		
Unnamed:			
From : Streptomyces RK 397	36	antitumoric	
From: Streptoverticillum MA-2664	37	antifungal	
From: Streptomyces CS-14	38	antifungal, antibacterial	
From : Streptomyces FR-008	39	antifungal, larvicidal	
From: Streptoverticillum 87-397			
Derived from Amphotericin B	40	antiviral (serine protease inhib)	
Just Numbers:			
17-41 b	1		
18-45	1		
58	1		
67-121 A	1		18
67-121 B	1		
67-121 C	1	antifungal	18
123	1		
131-1	1	antifungal	
243	1		
388	1		
500 1P	34	antifungal, antibacterial	
549-A1	1		
616	1		
661	1		
757	1		
991	1		
1008	1		
1033 A	1		
1033 B	1		
1645 P1	1		

References (Table 2 Polyene Macrolide Antibiotic Ligands)

1. Macrolide Antibiotics, edited by Satoshi Omura Academic Press, 1984
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WHAT IS CLAIMED IS:

1. A multibinding compound, or a salt thereof, comprising 2 to 10 polyene macrolide antibiotic ligands, which may be the same or different, covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane.

2. The multibinding compound of claim 1 wherein said ligand is selected from the group consisting of aureofuscin, rectilavendomycin, arenomycin B, lucensomycin, pimaricin, tetramycin A, tetramycin B, tetrin A, tetrin B, aurenin, rimocidin, fungichromin, elizabethin, chinin, filipin I, filipin II, filipin III, filipin IV, eurocidin A, eurocidin B, gannibamycin, mycoticin A, mycoticin B, rapamycin, demethoxyrapamycin, roflamycoin, dermostatin A, dermostatin B, nystatin A_1, nystatin A_3, polifungin B, amphotericin B, aureofungin A, candidin, hamycin, levorin A_2, mycoheptin, partricin A, partricin B, perimycin A, DJ-400-B_1, DJ-400-B_2, 67-121 A, 67-121-C, and lienomycin.

3. The multibinding compound of claim 1 wherein said ligand is amphotericin B.

4. The multibinding compound of claim 1 which has 2 ligands.

5. A multibinding compound of the formula:

$$(L)_p(X)_q$$

Formula I

or a salt thereof,

wherein each L is a polyene macrolide antibiotic ligand that may be the same or different at each occurrence, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane;
X is a linker that may be the same or different at each occurrence;
p is an integer of 2 to 10; and
q is an integer of 1 to 20.

6. The multibinding compound of claim 5 wherein *q* is less than *p*.

7. The multibinding compound of claim 5 wherein said ligand is selected from the group consisting of aureofuscin, rectilavendomycin, arenomycin B, lucensomycin, pimaricin, tetramycin A, tetramycin B, tetrin A, tetrin B, aurenin, rimocidin, fungichromin, elizabethin, chinin, filipin I, filipin II, filipin III, filipin IV, eurocidin A, eurocidin B, gannibamycin, mycoticin A, mycoticin B, rapamycin, demethoxyrapamycin, roflamycoin, dermostatin A, dermostatin B, nystatin A_1, nystatin A_3, polifungin B, amphotericin B, aureofungin A, candidin, hamycin, levorin A_2, mycoheptin, partricin A, partricin B, perimycin A, DJ-400-B_1, DJ-400-B_2, 67-121 A, 67-121-C, and lienomycin.

8. The multibinding compound of claim 5 wherein said ligand is amphotericin B.

9. The multibinding compound of claim 5 wherein p is 2 and q is 1.

10. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds, or pharmaceutically acceptable salts thereof, said

multibinding compound comprising 2 to 10 polyene macrolide antibiotic ligands, which may be the same or different, and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a membrane of a cell or virus mediating a pathologic condition in a mammal or plant.

11. The pharmaceutical composition of claim 10 wherein said ligand is selected from the group consisting of aureofuscin, rectilavendomycin, arenomycin B, lucensomycin, pimaricin, tetramycin A, tetramycin B, tetrin A, tetrin B, aurenin, rimocidin, fungichromin, elizabethin, chinin, filipin I, filipin II, filipin III, filipin IV, eurocidin A, eurocidin B, gannibamycin, mycoticin A, mycoticin B, rapamycin, demethoxyrapamycin, roflamycoin, dermostatin A, dermostatin B, nystatin A_1, nystatin A_3, polifungin B, amphotericin B, aureofungin A, candidin, hamycin, levorin A_2, mycoheptin, partricin A, partricin B, perimycin A, DJ-400-B_1, DJ-400-B_2, 67-121 A, 67-121-C, and lienomycin.

12. The pharmaceutical composition of claim 10 wherein said ligand is amphotericin B.

13. The pharmaceutical composition of claim 10 which has 2 ligands.

14. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of one or more multibinding compounds represented by the formula:

$$(L)_p(X)_q$$

Formula I

or a salt thereof,

wherein each L is a polyene macrolide antibiotic ligand that may be the same or different at each occurrence, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a cellular or viral membrane;
X is a linker that may be the same or different at each occurrence;
p is an integer of 2 to 10; and
q is an integer of 1 to 20.

15. The pharmaceutical composition of claim 14 wherein *q* is less than *p*.

16. The pharmaceutical composition of claim 14 wherein said ligand is selected from the group consisting of aureofuscin, rectilavendomycin, arenomycin B, lucensomycin, pimaricin, tetramycin A, tetramycin B, tetrin A, tetrin B, aurenin, rimocidin, fungichromin, elizabethin, chinin, filipin I, filipin II, filipin III, filipin IV, eurocidin A, eurocidin B, gannibamycin, mycoticin A, mycoticin B, rapamycin, demethoxyrapamycin, roflamycoin, dermostatin A, dermostatin B, nystatin A_1, nystatin A_3, polifungin B, amphotericin B, aureofungin A, candidin, hamycin, levorin A_2, mycoheptin, partricin A, partricin B, perimycin A, DJ-400-B_1, DJ-400-B_2, 67-121 A, 67-121-C, and lienomycin.

17. The pharmaceutical composition of claim 14 wherein said ligand is amphotericin B.

18. The pharmaceutical composition of claim 14 wherein *p* is 2 and *q* is 1.

19. A method for treating a pathologic condition of a mammal mediated by a cell or virus having a membrane binding site for a polyene macrolide antibiotic, which method comprises administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds, or pharmaceutically acceptable salts thereof, comprising 2 to 10 polyene macrolide antibiotic ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a ligand binding site in a membrane of said cell or virus thereby inhibiting the pathologic condition.

20. The method of claim 19 wherein said ligand is selected from the group consisting of aureofuscin, rectilavendomycin, arenomycin B, lucensomycin, pimaricin, tetramycin A, tetramycin B, tetrin A, tetrin B, aurenin, rimocidin, fungichromin, elizabethin, chinin, filipin I, filipin II, filipin III, filipin IV, eurocidin A, eurocidin B, gannibamycin, mycoticin A, mycoticin B, rapamycin, demethoxyrapamycin, roflamycoin, dermostatin A, dermostatin B, nystatin A_1, nystatin A_3, polifungin B, amphotericin B, aureofungin A, candidin, hamycin, levorin A_2, mycoheptin, partricin A, partricin B, perimycin A, DJ-400-B_1, DJ-400-B_2, 67-121 A, 67-121-C, and lienomycin.

21. The method of claim 19 wherein said ligand is amphotericin B.

22. The method of claim 19 which has 2 ligands.

23. The method of claim 19 wherein the pathologic condition is caused by a fungal infection.

24. A method for treating a pathologic condition of a mammal mediated by a cell or virus having a membrane binding site for a polyene macrolide antibiotic, which method comprises administering to said mammal or plant an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by the formula:

$(L)_p(X)q$

Formula I

or a salt thereof,

wherein each L is a polyene macrolide antibiotic ligand that may be the same or different at each occurrence, each of said ligands comprising a ligand domain capable of binding to a ligand binding site cellular or viral membrane;

X is a linker that may be the same or different at each occurrence;

p is an integer of 2 to 10; and

q is an integer of 1 to 20.

25. The method of claim 24 wherein *q* is less that *p*.

26. The method of claim 24 wherein said ligand is selected from the group consisting of aureofuscin, rectilavendomycin, arenomycin B, lucensomycin, pimaricin, tetramycin A, tetramycin B, tetrin A, tetrin B, aurenin, rimocidin, fungichromin, elizabethin, chinin, filipin I, filipin II, filipin III, filipin IV, eurocidin A, eurocidin B, gannibamycin, mycoticin A, mycoticin B, rapamycin, demethoxyrapamycin, roflamycoin, dermostatin A, dermostatin B, nystatin A_1, nystatin A_3, polifungin B, amphotericin B, aureofungin A, candidin, hamycin,

levorin A_2, mycoheptin, partricin A, partricin B, perimycin A, DJ-400-B_1, DJ-400-B_2, 67-121 A, 67-121-C, and lienomycin.

27. The method of claim 24, wherein said ligand is amphotericin B.

28. The method of claim 24 wherein *p* is 2 and *q* is 1.

29. The method of claim 24 wherein the pathologic condition is caused by a fungal infection.

30. A method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

31. A method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

32. The method according to Claim 30 or 31 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

33. The method according to Claim 32 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

34. The method according to Claim 33 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

35. The method according to Claim 34 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

36. The method according to Claim 30or 31wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

37. The method according to Claim 36 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

38. The method according to Claim 30 or 31 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

39. The method according to Claim 38 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

40. The method according to Claim 39 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

41. The method according to Claim 30 or 31 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

42. The method according to Claim 41 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

43. The method according to Claim 30 or 31 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

44. The method according to Claim 30 or 31 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

45. The method according to Claim 30 or 31 wherein at least one of the ligands is capable of binding to a ligand binding site in a cellular or viral membrane.

46. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified

in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

47. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

48. The library according to Claim 46 or 47 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

49. The library according to Claim 48 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

50. The library according to Claim 49 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

51. The library according to Claim 46 or 47 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

52. The library according to Claim 51 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

53. The library according to Claim 46 or 48 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

54. The library according to Claim 46 or 47 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

55. The method according to Claims 46 or 47 wherein at least one of the ligands is capable of binding to a ligand binding site in a cellular or viral membrane.

56. An iterative method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

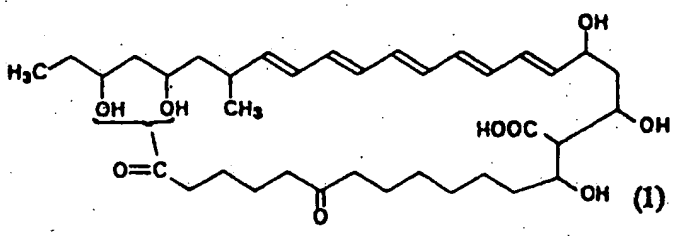
(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

57. The method according to Claim 56 wherein steps (e) and (f) are repeated from 2-50 times.

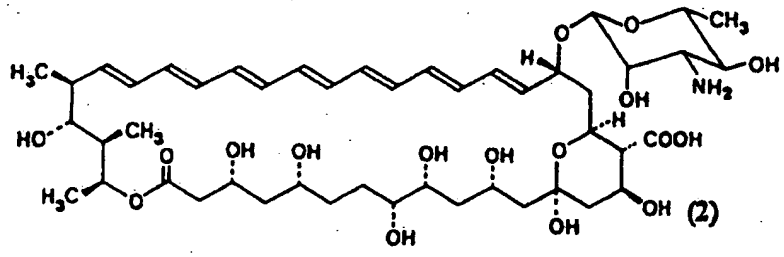
58. The method according to Claim 56 wherein steps (e) and (f) are repeated from 5-50 times.

59. The method according to Claim 56 wherein at least one of the ligands is capable of binding to a ligand binding site in a cellular or viral membrane.

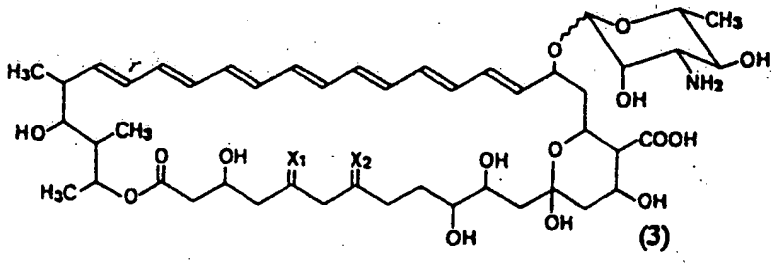
FIGURE 1



Aglycone of gannibamycin
(partial structure)

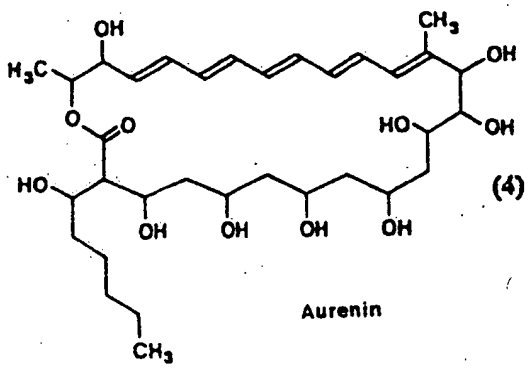


Amphotericin B



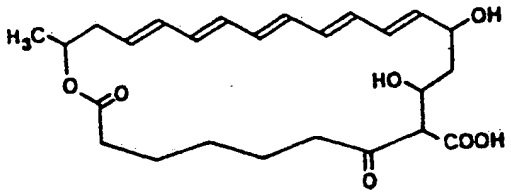
	X_1	X_2
Candidin	H, OH	=O
Mycoheptin	=O	H, OH

FIGURE 1, continued



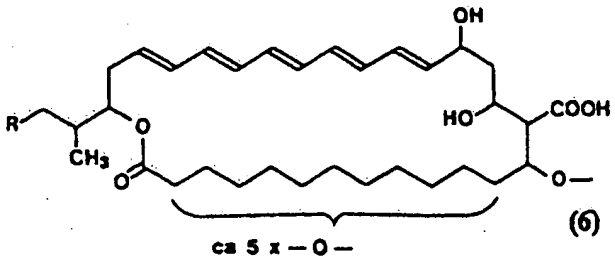
(4)

Aurenin



(5)

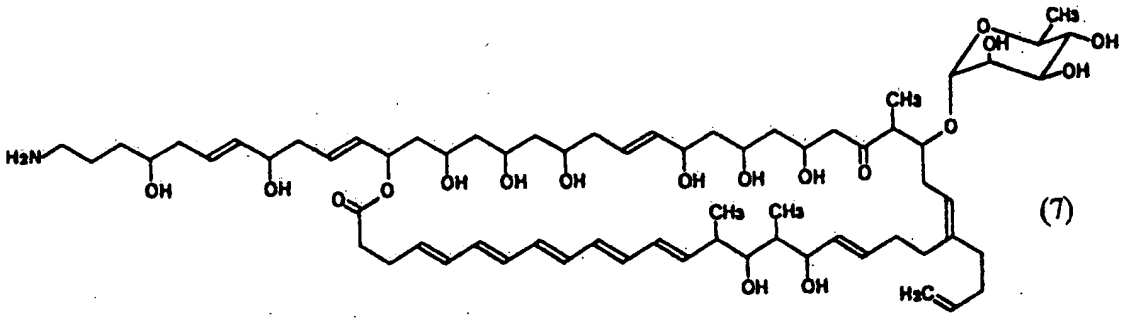
Aglycone of rectilavendomycin



(6)

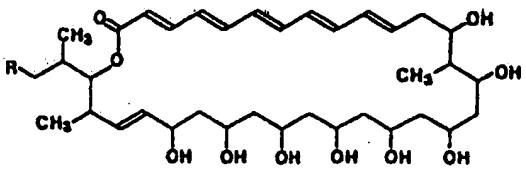
Aglycone of eurocidin A R=CH_3
Aglycone of eurocidin B R=H
(partial structure)

FIGURE 1, continued



(7)

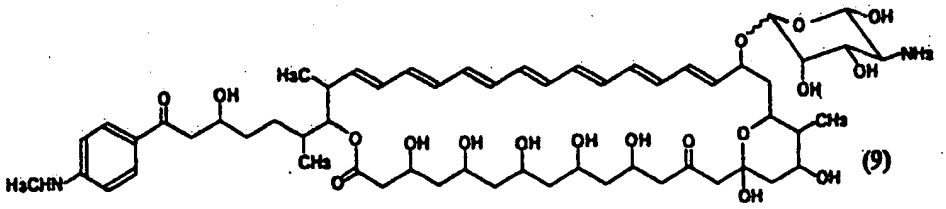
Lienomycin



(8)

Mycoticin A R = H
(major component of flavofungin)

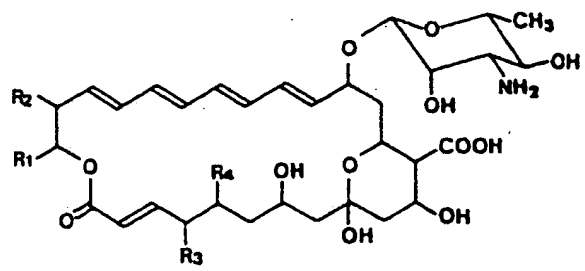
Mycoticin B R = CH_3
(minor component of flavofungin)



(9)

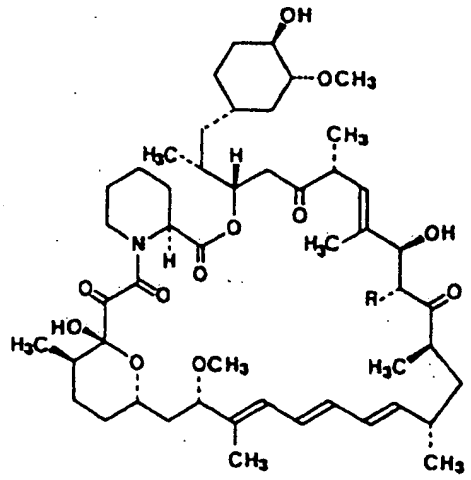
Perimycin A

FIGURE 1, continued



(10)

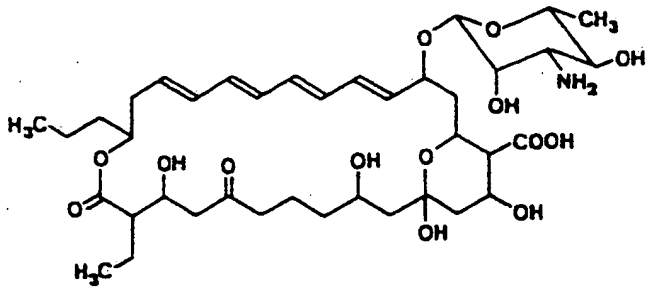
	R_1	R_2	R_3	R_4
Pimaricin	CH_3	H	– O – (epoxy)	
Lucensomycin	n–C_4H_9	H	– O – (epoxy)	
Arenomycin B	n–C_4H_9	H	H	OH
Tetrin A	CH_3	CH_3	H	OH
Tetrin B	CH_3	CH_3	OH	OH
Tetramycin A	CH_3	C_2H_5	H	OH
Tetramycin B	CH_3	C_2H_5	OH	OH



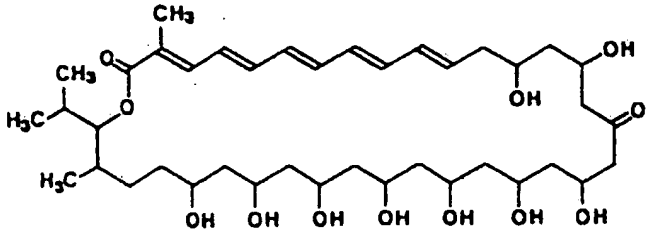
(11)

Rapamycin	R = CH_3O
Demethoxyrapamycin	R = H

FIGURE 1, continued

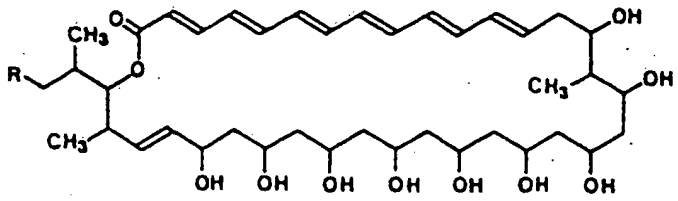


Rimocidin (12)



Roflamycoin (flavomycoin)

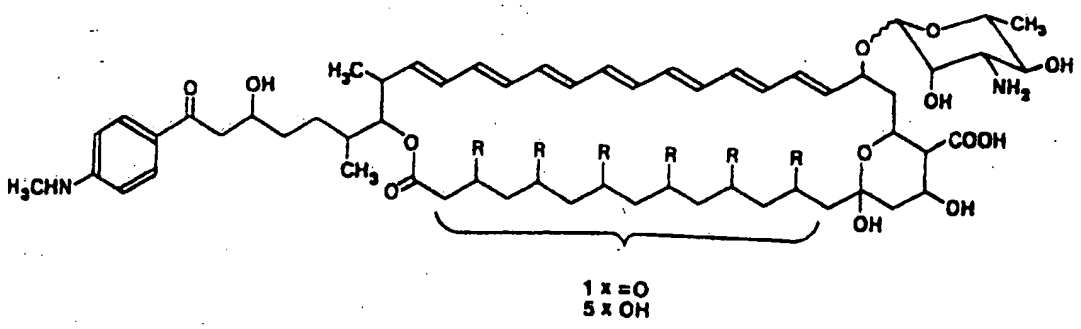
(13)



Dermostatin A R=H
Dermostatin B R=CH_3

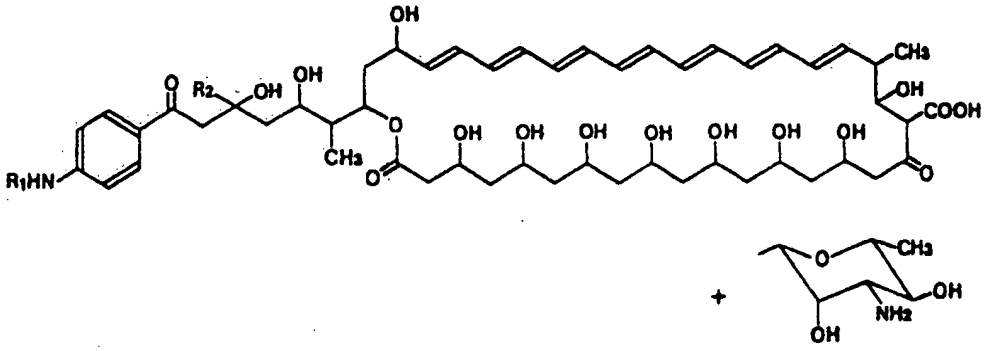
(14)

FIGURE 1. continued



(15)

Partial structure of aureofungin A

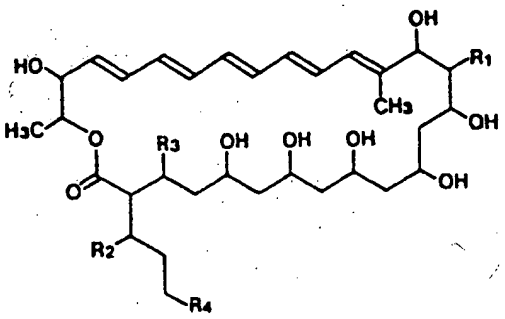


DJ-400 B_1 R_1 = CH_3
R_2 =

DJ-400 B_2 R_1 = R_2 = H

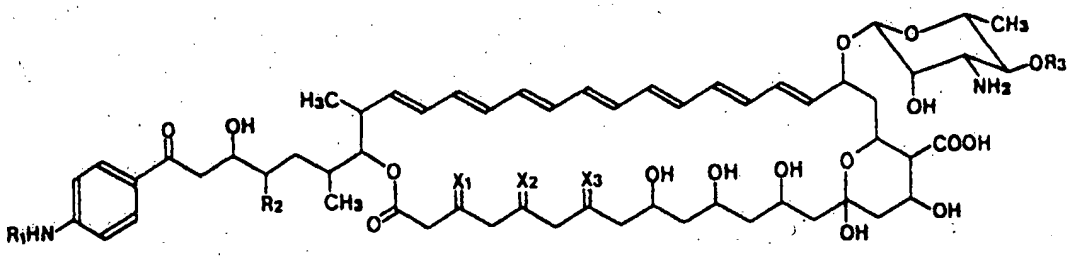
(16)

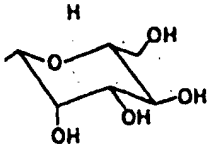
FIGURE 1, continued



	R_1	R_2	R_3	R_4
Fungichromin (lagosin, cogomycin)	OH	OH	OH	C_3H_7
Elizabethin	stereoisomer of fungichromin			
Chainin	H	H	OH	CH_3
Filipin I	dideoxy-filipin III			
Filipin II	deoxy-filipin III			
Filipin III (filipin)	H	OH	OH	C_3H_7
Filipin IV	stereoisomer of filipin III			

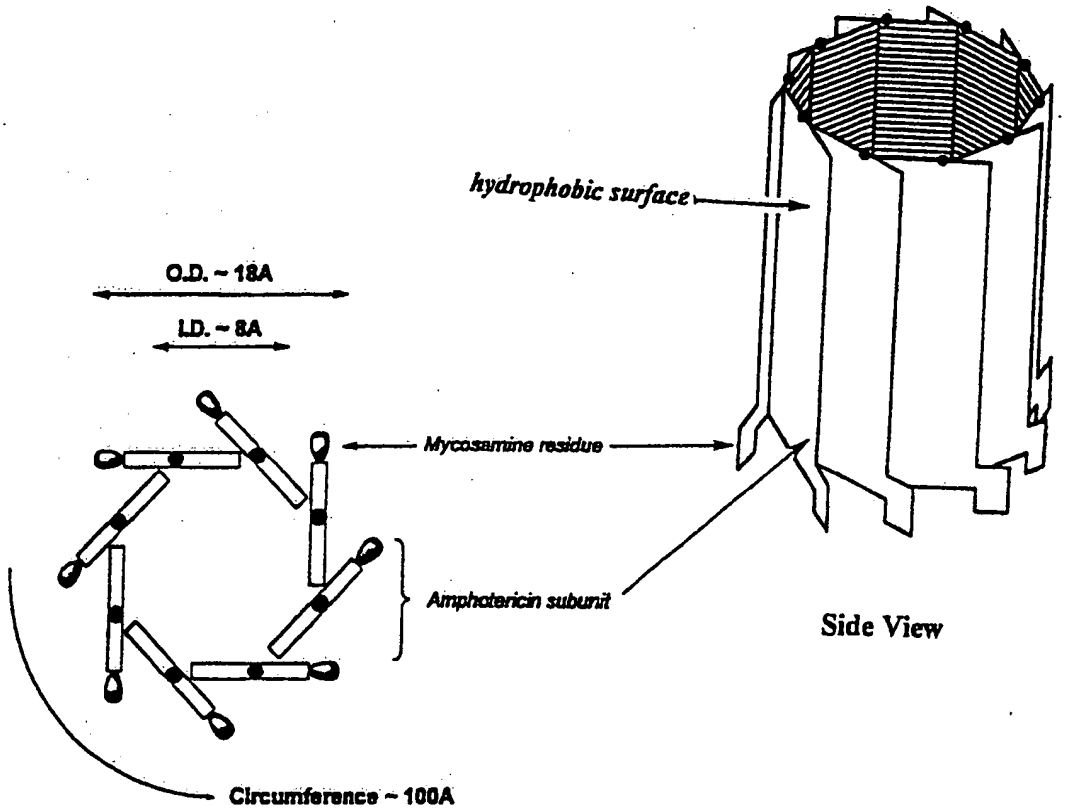
(17)



	R_1	R_2	R_3	X_1	X_2	X_3
Hamycin	H	H	H	H,OH	H,OH	H,OH
Levorin A2 (candicidin D)	H	CH_3	H	=O	H,H	=O
Partricin A	CH_3	H	H	H,OH	=O	H,OH
Partricin B (vacidin A)	H	H	H	H,OH	=O	H,OH
67-121A	CH_3	H	H	H,OH	H,OH	H,OH
67-121C	CH_3	H		H,OH	H,OH	H,OH

(18)

FIGURE 2



Cross-Sectional View

Figure 3

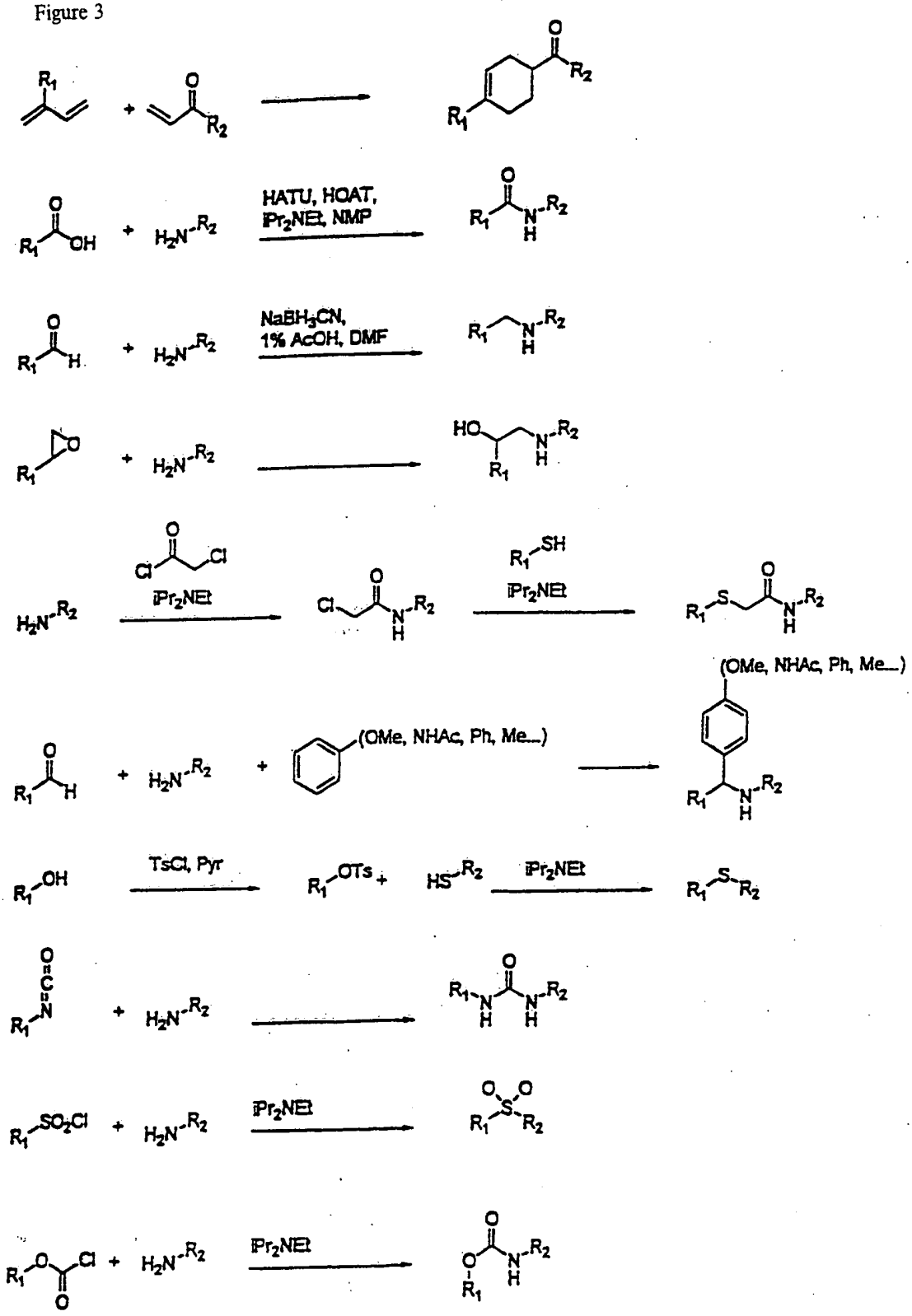
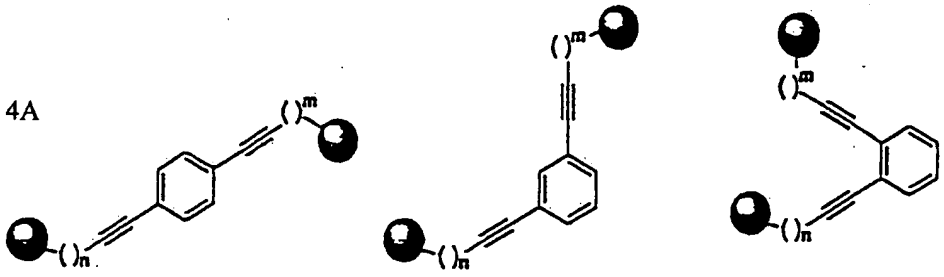

HATU, HOAT,
iPr2NEt, NMP
NaBH3CN,
1% AcOH, DMF
iPr2NEt
iPr2NEt
(OMe, NHAc, Ph, Me...)
(OMe, NHAc, Ph, Me...)
TsCl, Pyr
iPr2NEt
iPr2NEt
iPr2NEt

Figure 4A



= ligand

n+m+core<100 atoms

Figure 4B

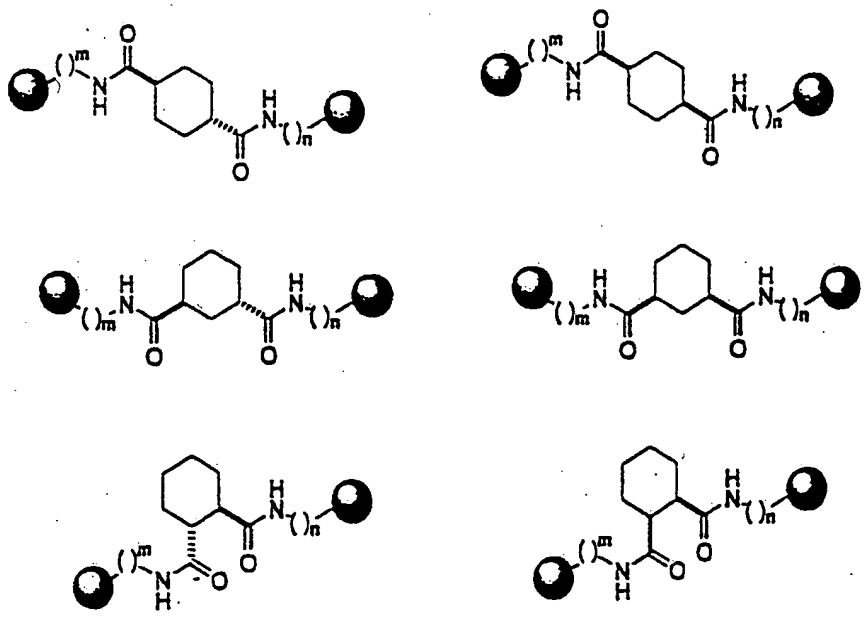


Figure 5

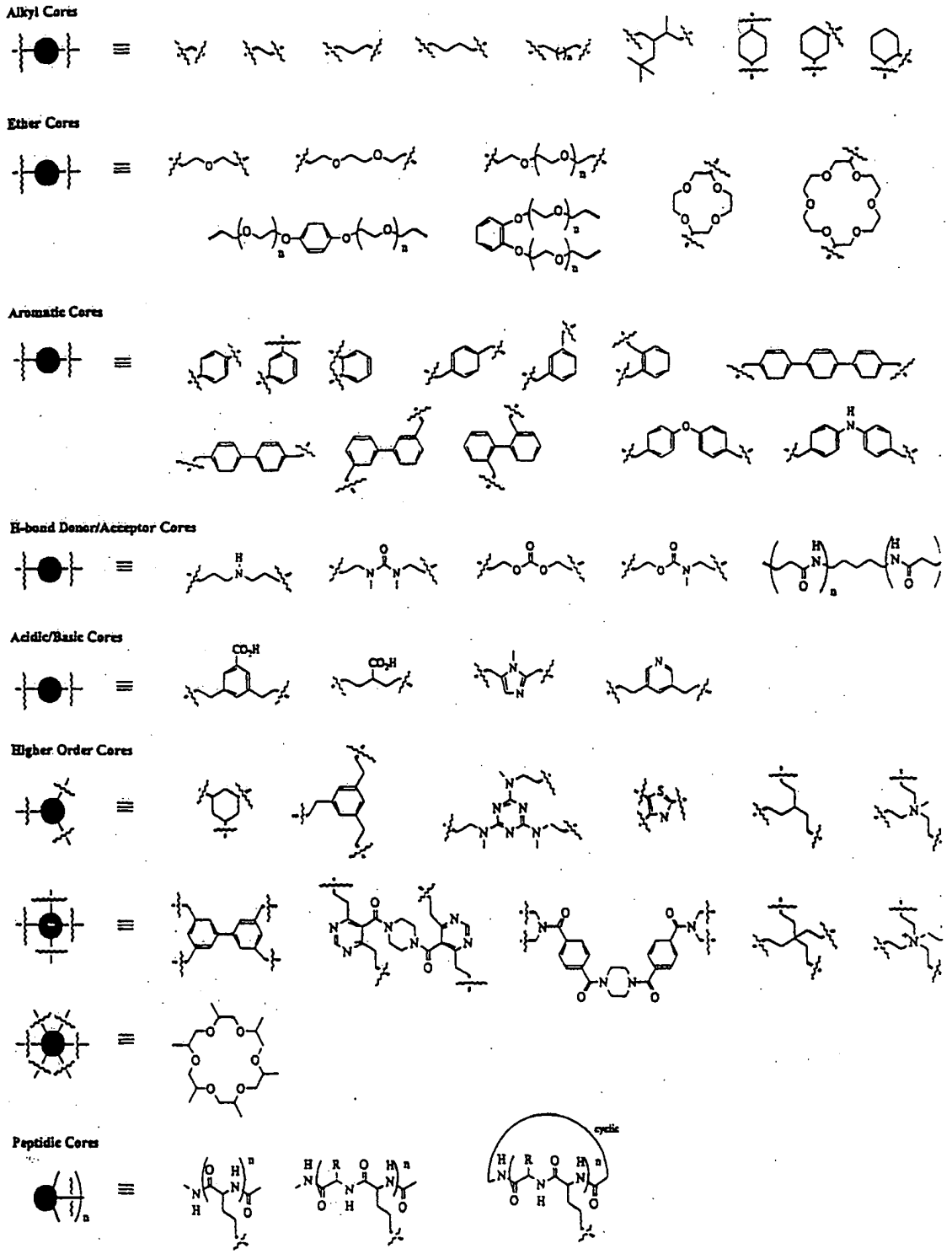

Alkyl Cores
Ether Cores
Aromatic Cores
H-bond Donor/Acceptor Cores
Acidic/Basic Cores
Higher Order Cores
Peptidic Cores
cyclic

Figure 6A

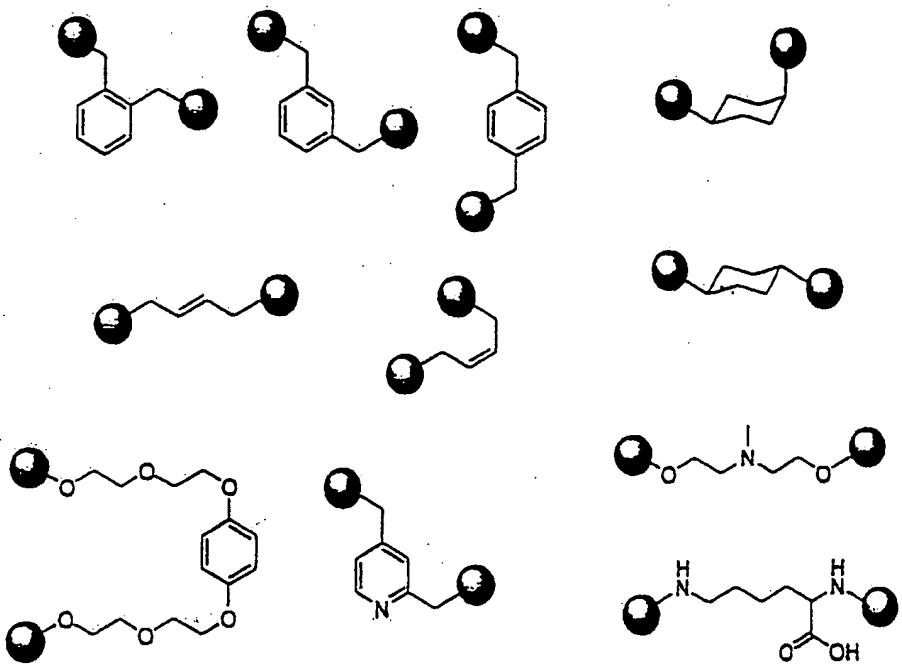


Figure 6B

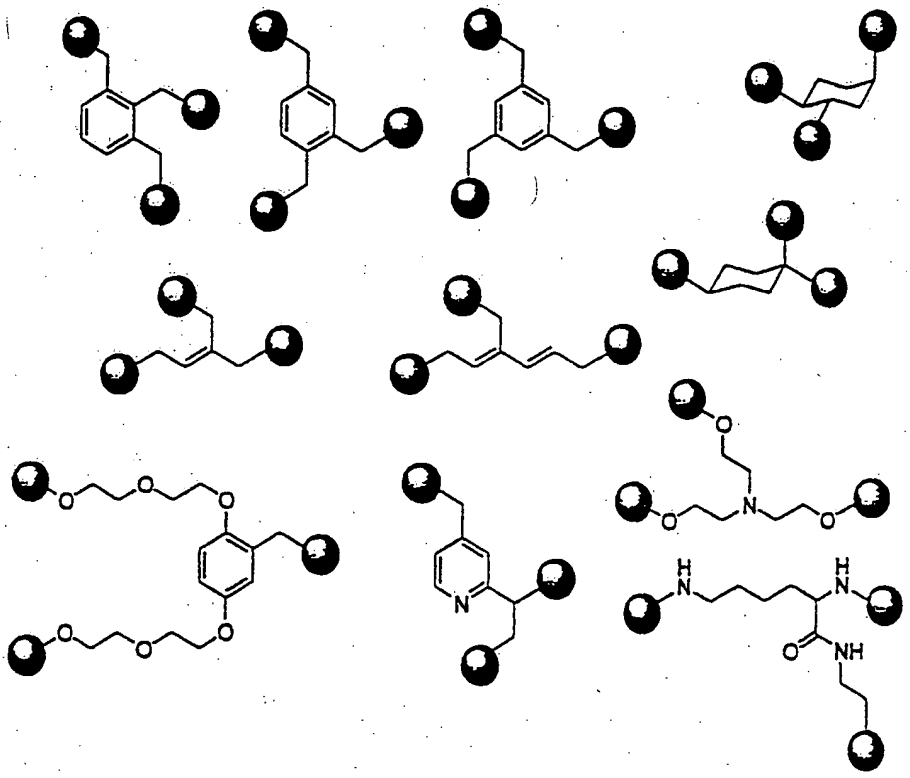


Figure 6C

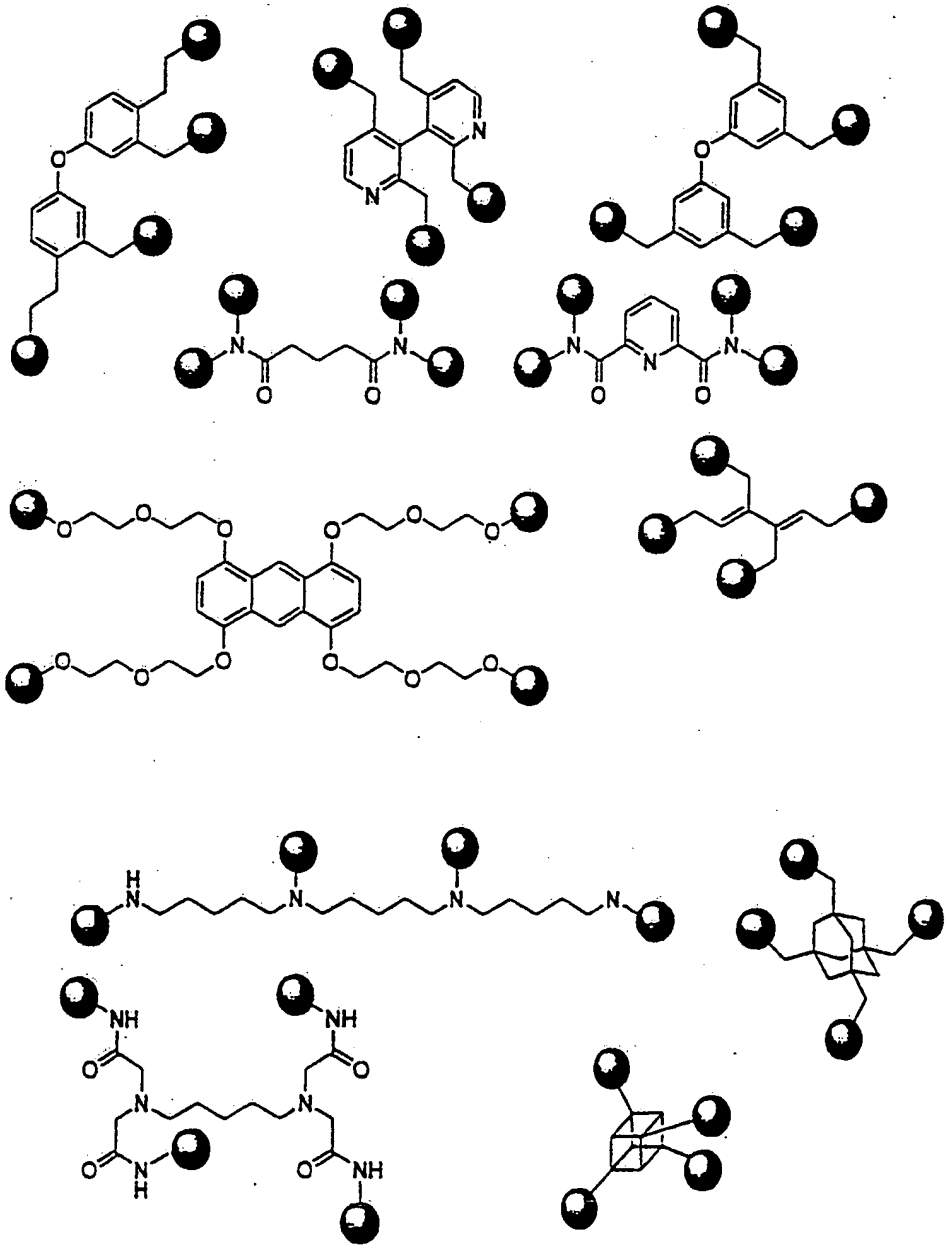
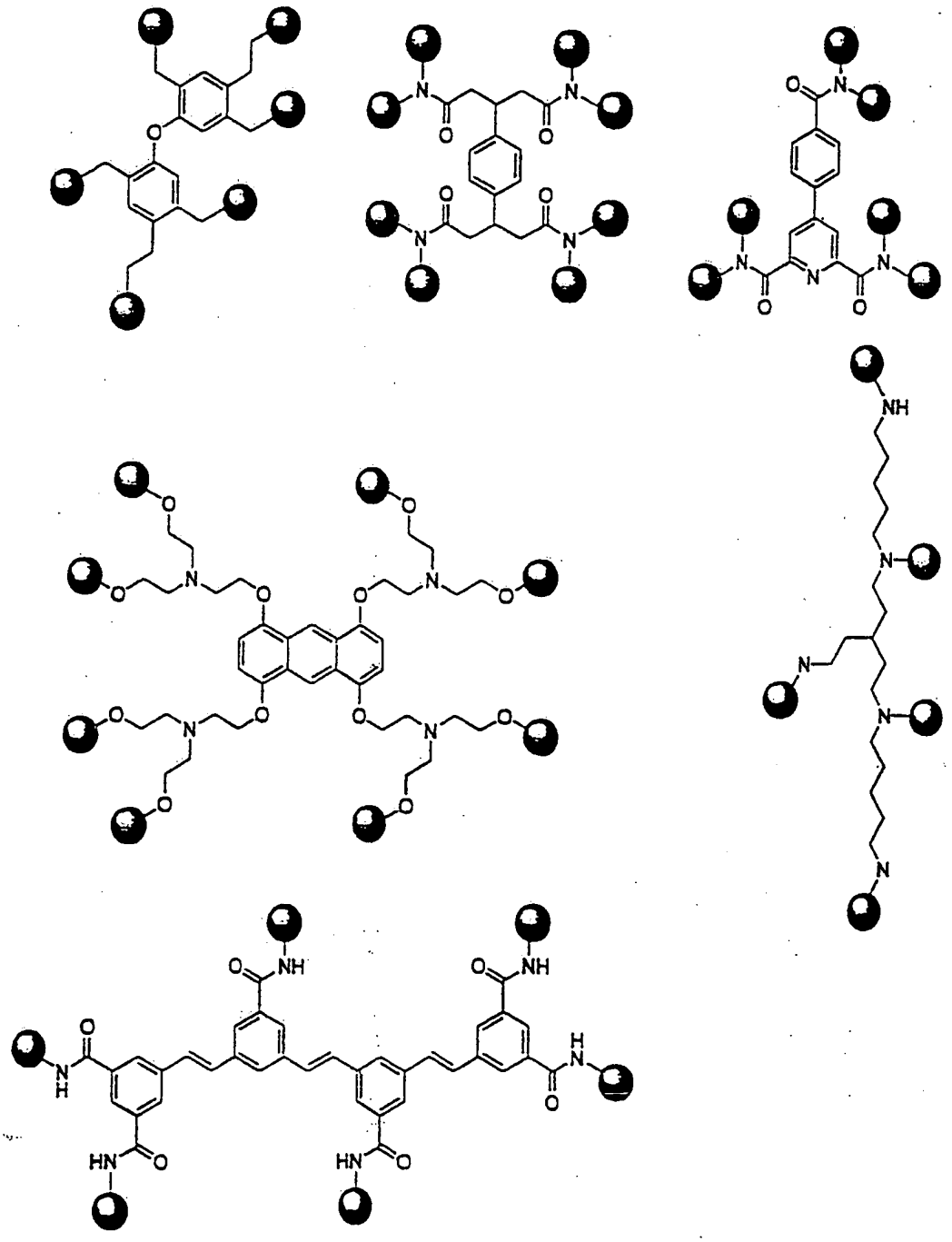
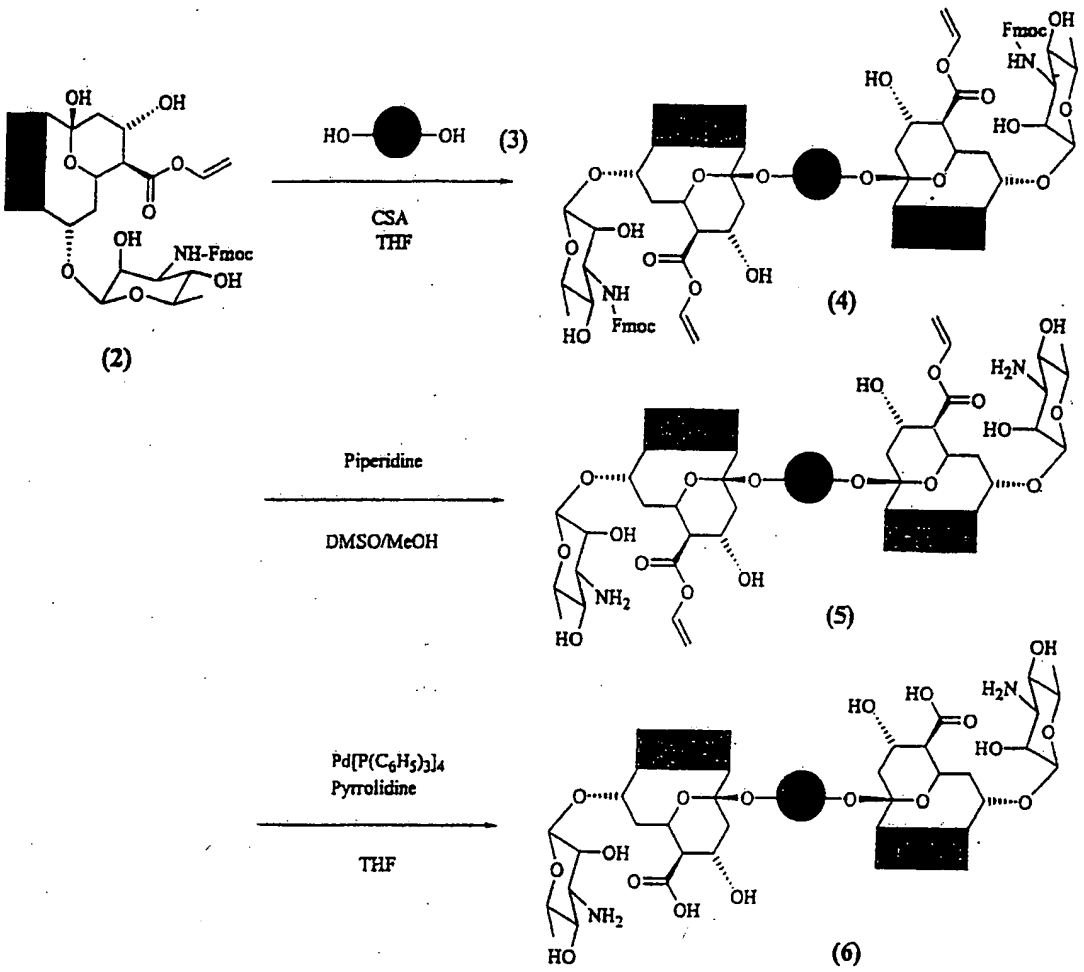


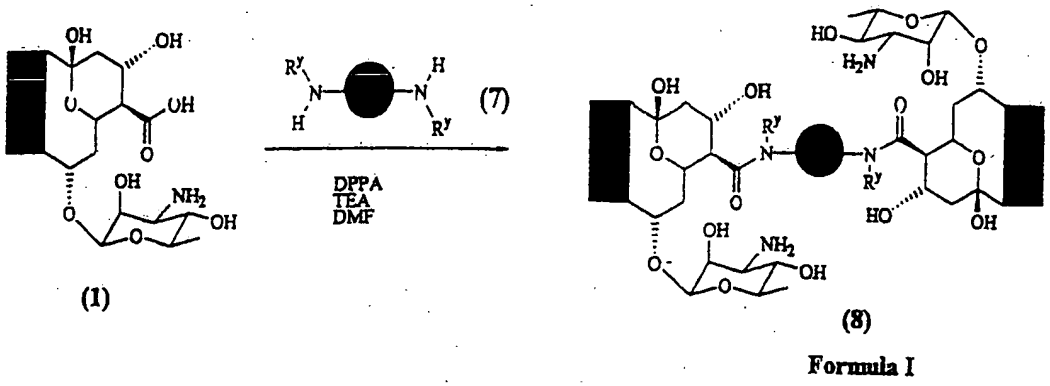
Figure 6D





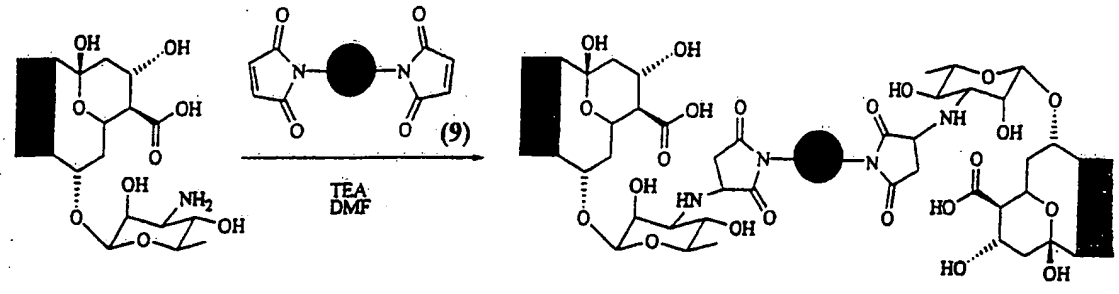
Formula I

Figure 7



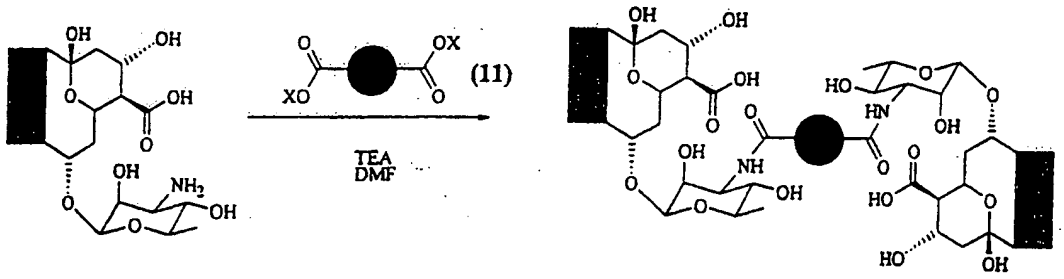
Formula I

Figure 8



(1) (10)

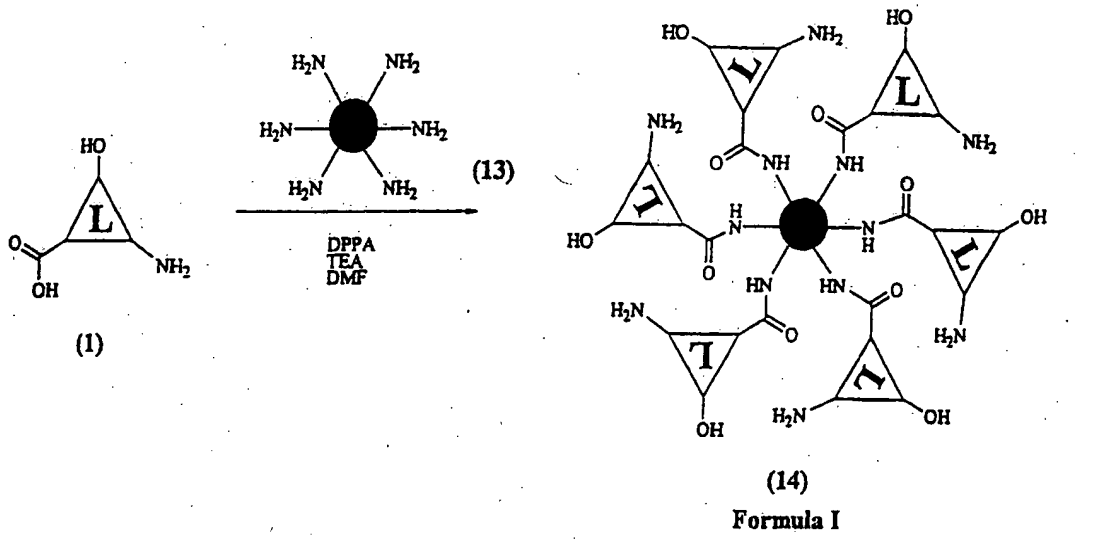
Formula I



(1) (12)

Formula I

Figure 9



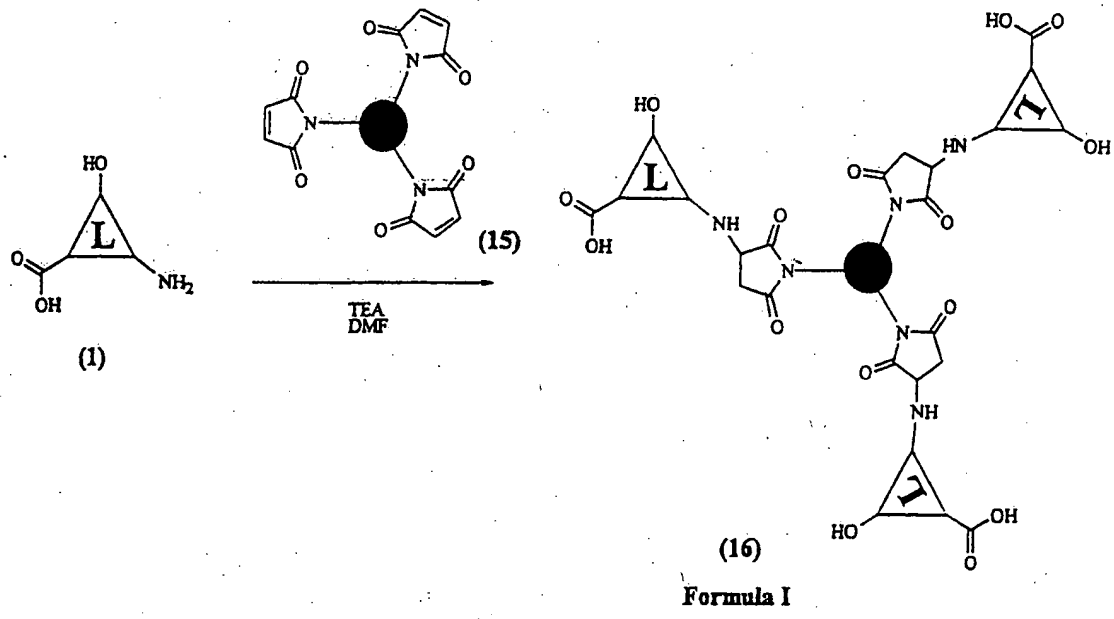
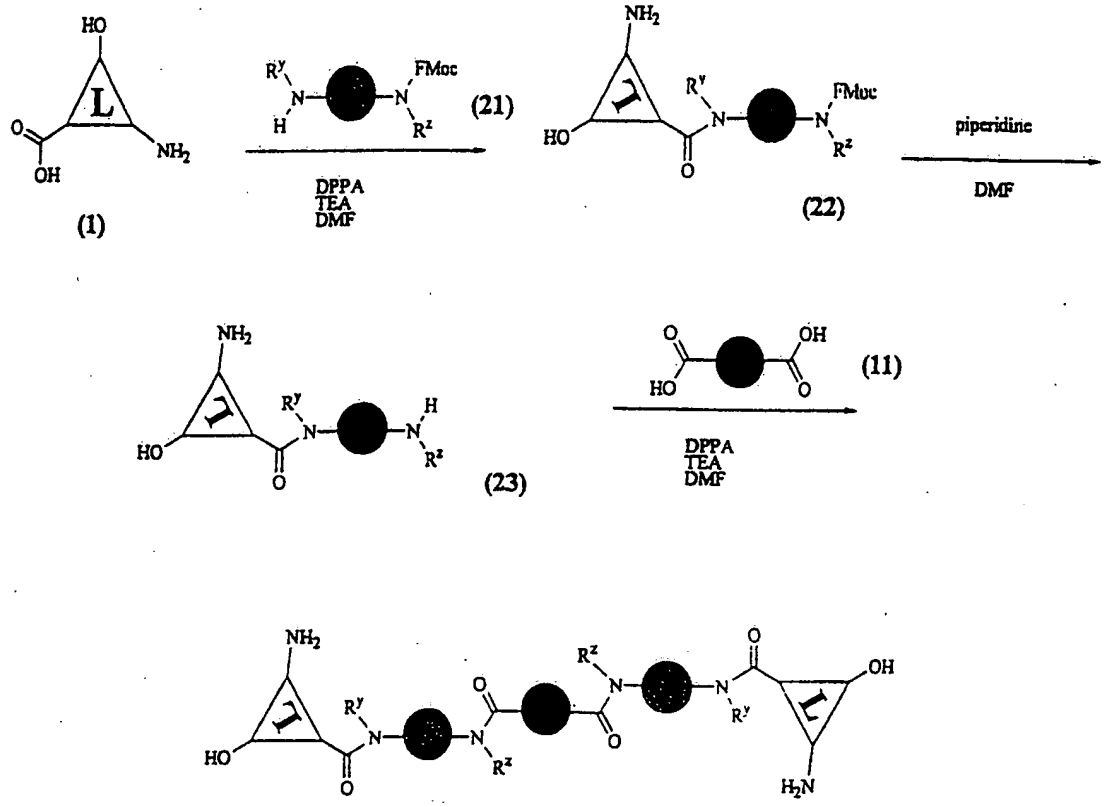
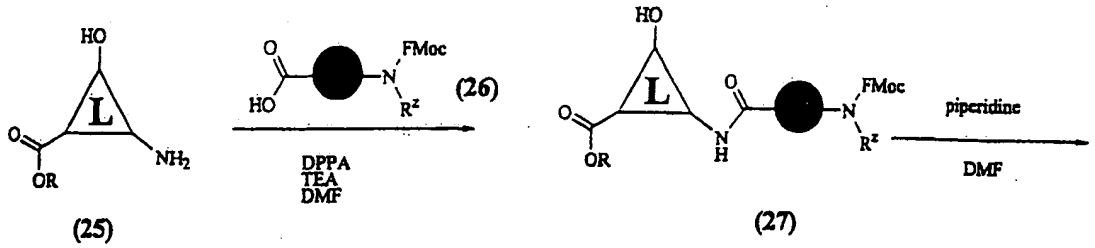


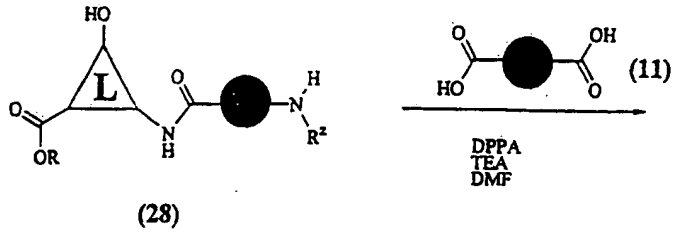
Figure 10

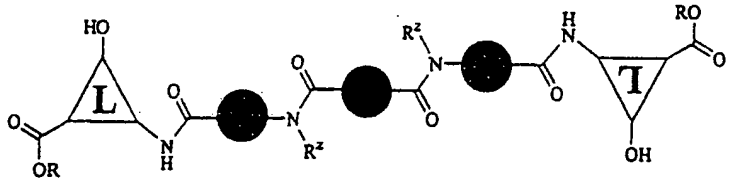


Formula I

Figure 11







(29)

Formula I

Figure 12

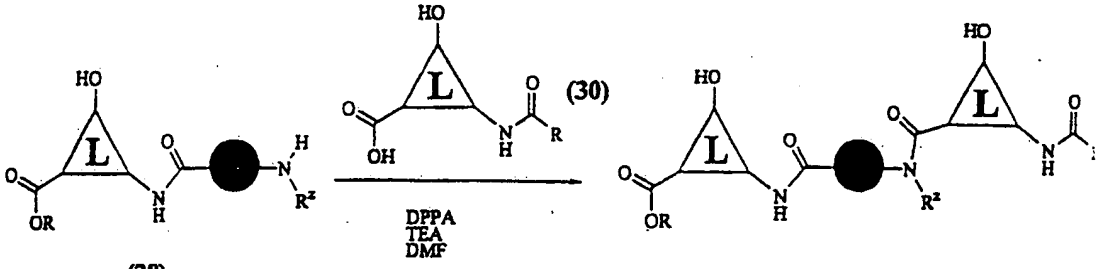
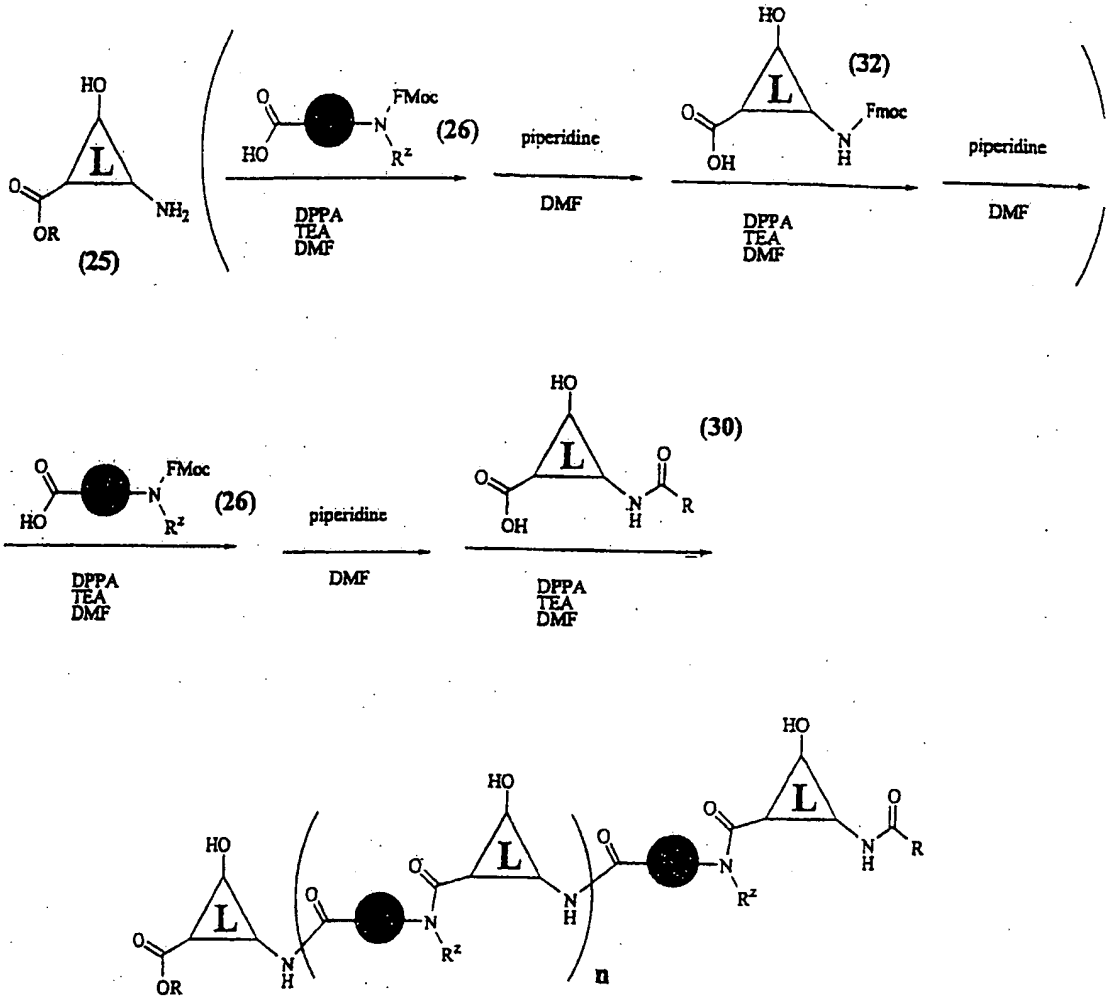
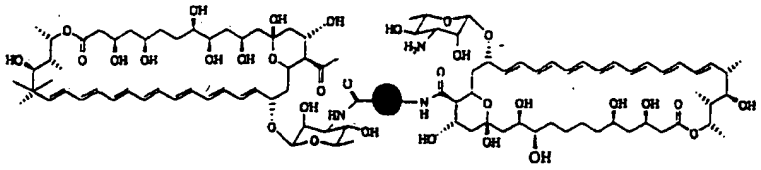

HO
L
O
OR
N
H
O
N
H
R²
(28)
HO
L
O
OH
O
N
H
R
(30)
DPPA
TEA
DMF
HO
L
O
OR
N
H
O
N
R²
HO
L
O
N
H
(31)
Formula I

Figure 13

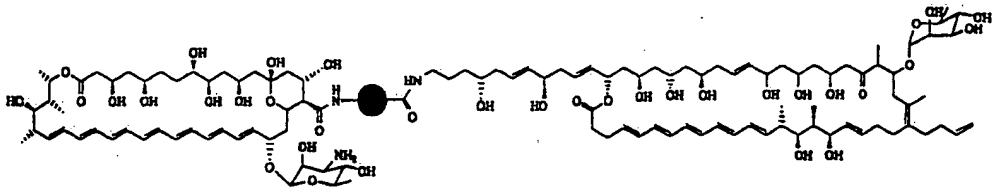


Formula I

Figure 14



Amphotericin B - Nystatin



Amphotericin B - Lienomycin

Figure 15

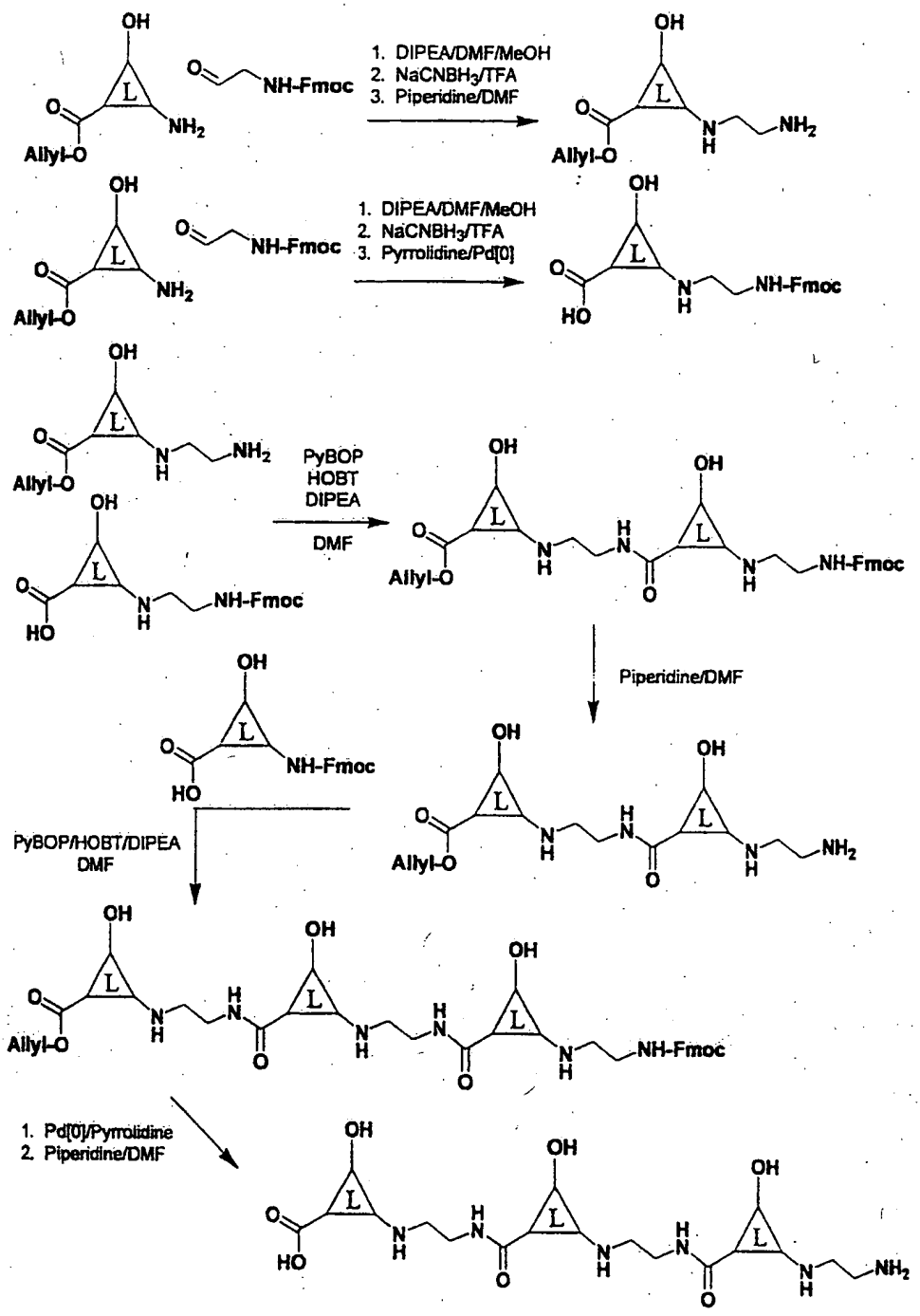

1. DIPEA/DMF/MeOH
2. NaCNBH3/TFA
3. Piperidine/DMF
1. DIPEA/DMF/MeOH
2. NaCNBH3/TFA
3. Pyrrolidine/Pd[0]
PyBOP
HOBT
DIPEA
DMF
Piperidine/DMF
PyBOP/HOBT/DIPEA
DMF
1. Pd[0]/Pyrrolidine
2. Piperidine/DMF

Figure 16

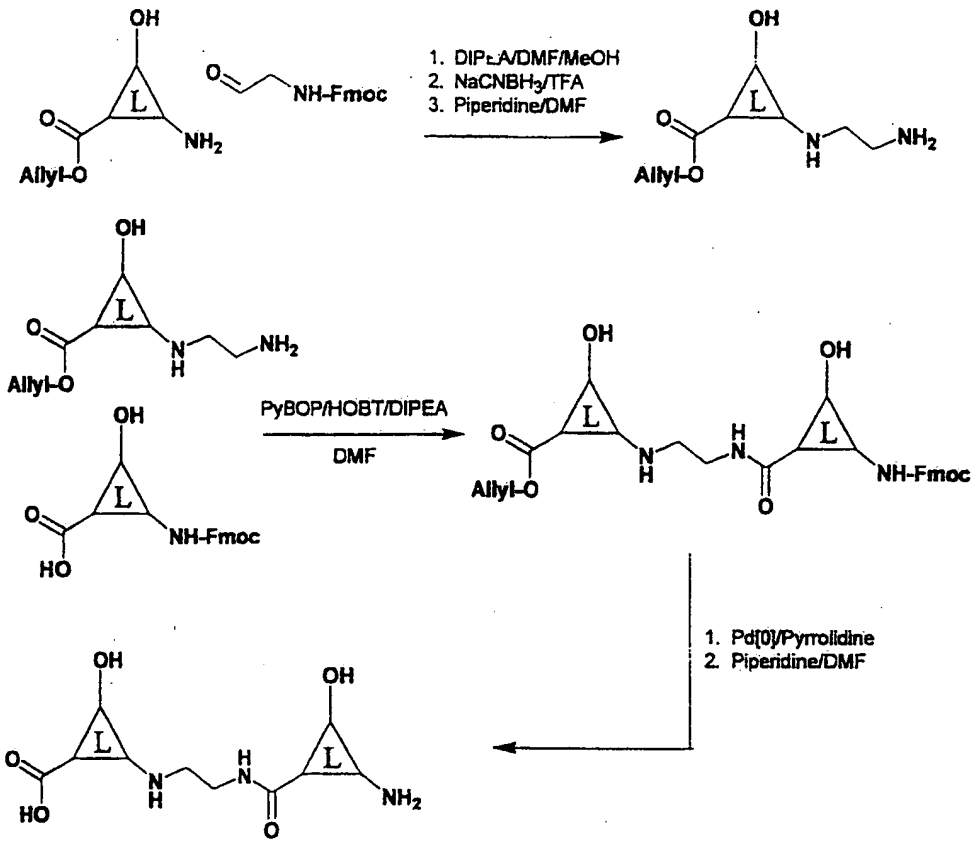

OH
L
O
NH2
Allyl-O
O
NH-Fmoc
1. DIPEA/DMF/MeOH
2. NaCNBH3/TFA
3. Piperidine/DMF
N
H
PyBOP/HOBT/DIPEA
DMF
NH-Fmoc
HO
1. Pd[0]/Pyrrolidine
2. Piperidine/DMF

Figure 17

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CORRECTED VERSION

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International Bureau

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(25) Filing Language: English

(26) Publication Language: English

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60/097,698	25 August 1998 (25.08.1998)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/092,941 (CON)
Filed on	15 July 1998 (15.07.1998)
US	60/088,464 (CON)
Filed on	8 June 1998 (08.06.1998)
US	60/097,698 (CON)
Filed on	25 August 1998 (25.08.1998)

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(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US). JUDICE, J., Kevin [US/US]; 212 Dolores Street, El Granada, CA 94018 (US).

(74) Agents: SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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WO 99/64040 A1

(54) Title: NOVEL POLYENE MACROLIDE COMPOUNDS AND USES

(57) Abstract: Novel multibinding compounds are disclosed. The compounds of the invention comprise 2-10 polyene macrolide antibiotic ligands covalently connected, each of said ligands being capable of binding to a ligand binding site in a cellular or viral membrane, thereby modulating the biological processes/functions thereof.